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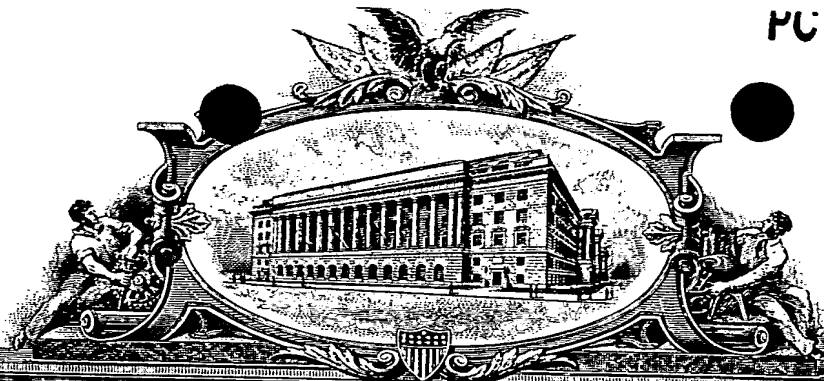
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June 22, 2000

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 09/350,982

FILING DATE: July 09, 1999

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN
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☐ A Provisional Patent Application under 37 C.F.R. 1.53(c).

☐ A Design Patent Application (submitted in duplicate).

Including the following:

☐ Provisional Application Cover Sheet.

☒ New or Revised Specification, including pages 1 to 40 containing:

☒ Specification

☒ Claims

☒ Abstract

☐ Substitute Specification, including Claims and Abstract.

☐ The present application is a continuation application of Application No. _____ filed _____. The present application includes the Specification of the parent application which has been revised in accordance with the amendments filed in the parent application. Since none of those amendments incorporate new matter into the parent application, the present revised Specification also does not include new matter.

☐ The present application is a continuation application of Application No. _____ filed _____, which in turn is a continuation-in-part of Application No. _____ filed _____. The present application includes the Specification of the parent application which has been revised in accordance with the amendments filed in the parent application. Although the amendments in the parent C-I-P application may have incorporated new matter, since those are the only revisions included in the present application, the present application includes no new matter in relation to the parent application.

☐ A copy of earlier application Serial No. _____ Filed _____, including Specification, Claims and Abstract (pages 1 - @@), to which no new matter has been added TOGETHER WITH a copy of the executed oath or declaration for such earlier application and all drawings and appendices. Such earlier application is hereby incorporated into the present application by reference.

☐ Please enter the following amendment to the Specification under the Cross-Reference to Related Applications section (or create such a section) : "This Application:

☐ is a continuation of ☐ is a divisional of ☐ claims benefit of U.S. provisional Application Serial No. _____ filed _____

- ☐ Signed Statement attached declaring inventor(s) named in the prior application.
- ☐ A Preliminary Amendment.
- ☐ _____ Sheets of ☐ Formal ☐ Informal Drawings.
- ☐ Petition to Accept Photographic Drawings.
- ☐ Petition Fee
- ☒ An ☐ Executed ☒ Unexecuted Declaration or Oath and Power of Attorney.
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- ☐ An ☐ Executed ☐ Copy of Executed Assignment of the Invention to _____
- ☐ A Recordation Form Cover Sheet.
- ☐ Recordation Fee - \$40.00.
- ☐ The prior application is assigned of record to _____
- ☐ Priority is claimed under 35 U.S.C. § 119 of Patent Application No. _____
filed _____ in _____ (country).
- ☐ A Certified Copy of each of the above applications for which priority is claimed:
- ☐ is enclosed.
- ☐ has been filed in prior application Serial No. _____ filed _____.
- ☐ An ☐ Executed or ☐ Copy of Executed Earlier Statement Claiming Small Entity Status under 37 C.F.R. 1.9 and 1.27
- ☐ is enclosed.
- ☐ has been filed in prior application Serial No. _____ filed _____, said status is still proper and desired in present case.

- ☒ Diskette Containing DNA/Amino Acid Sequence Information.
- ☒ Statement to Support Submission of DNA/Amino Acid Sequence Information.
- ☐ The computer readable form in this application _____, is identical with that filed in Application Serial Number _____, filed _____. In accordance with 37 CFR 1.821(e), please use the ☐ first-filed, ☐ last-filed or ☐ only computer readable form filed in that application as the computer readable form for the instant application. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the computer readable form that will be used for the instant application. A paper copy of the Sequence Listing is ☐ included in the originally-filed specification of the instant application, ☐ included in a separately filed preliminary amendment for incorporation into the specification.
- ☐ Information Disclosure Statement.
- ☐ Attached Form 1449.
- ☐ Copies of each of the references listed on the attached Form PTO-1449 are enclosed herewith.
- ☐ A copy of Petition for Extension of Time as filed in the prior case.
- ☐ Appended Material as follows: _____
- ☒ Return Receipt Postcard (should be specifically itemized).
- ☐ Other as follows: _____

FEE CALCULATION:

- ☐ Cancel in this application original claims _____ of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

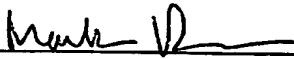
				SMALL ENTITY		NOT SMALL ENTITY	
				RATE	FEE	RATE	FEE
PROVISIONAL APPLICATION				\$75.00	\$	\$150.00	\$
DESIGN APPLICATION				\$155.00	\$	\$310.00	\$
UTILITY APPLICATIONS BASE FEE				\$380.00	\$	\$760.00	\$760.00
UTILITY APPLICATION; ALL CLAIMS CALCULATED AFTER ENTRY OF ALL AMENDMENTS							
	No. Filed	No. Extra					
TOTAL CLAIMS	77- 20 =	57	\$9 each	\$	\$18 each	\$1026.00	
INDEP. CLAIMS	6- 3 =	3	\$39 each	\$	\$78 each	\$234.00	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				\$130	\$	\$260	\$130.00
ADDITIONAL FILING FEE					\$		\$0
TOTAL FILING FEE DUE					\$		\$2150

- ☒ A Check is enclosed in the amount of \$ 2150.00.
- ☒ The Commissioner is authorized to charge payment of the following fees and to refund any overpayment associated with this communication or during the pendency of this application to deposit account 23-3050. This sheet is provided in duplicate.
- ☐ The foregoing amount due.
- ☒ Any additional filing fees required, including fees for the presentation of extra claims under 37 C.F.R. 1.16.
- ☒ Any additional patent application processing fees under 37 C.F.R. 1.17 or 1.20(d).
- ☐ The issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance.
- ☒ The Commissioner is hereby requested to grant an extension of time for the appropriate length of time, should one be necessary, in connection with this filing or any future filing submitted to the U.S. Patent and Trademark Office in the above-

identified application during the pendency of this application. The Commissioner is further authorized to charge any fees related to any such extension of time to deposit account 23-3050. This sheet is provided in duplicate.

SHOULD ANY DEFICIENCIES APPEAR with respect to this application, including deficiencies in payment of fees, missing parts of the application or otherwise, the United States Patent and Trademark Office is respectfully requested to promptly notify the undersigned.

Date: July 9, 1999



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TANKYRASE HOMOLOG PROTEIN (THP), NUCLEIC ACIDS, AND METHODS RELATED TO THE SAME

FIELD OF THE INVENTION

5 The present invention is directed, in part, to nucleic acid molecules encoding tankyrase homolog protein (THP), novel THP polypeptides, and assays for screening compounds which bind to THP and/or modulate the activity of THP.

BACKGROUND OF THE INVENTION

10 Telomeres, the physical ends of eukaryotic chromosomes, consist of short repeated nucleotide sequences that are characteristic for all telomeric DNA, from yeast to human (see, e.g., Harley *et al.*, *Curr. Opin. Genet. Dev.*, 1995, 5, 249). Telomeres are essential for the integrity of chromosomes (see, e.g., Lundblad *et al.*, *Cell*, 1996, 87, 369), since DNA polymerases are incapable of replicating the very end of DNA. This
15 replication defect affects the telomeres which are progressively shortened with each cell cycle, and as they reach a critical short length, cells will die (see, e.g., Goldstein, *Science*, 1990, 249, 1129). In some cells, telomere length is preserved by a ribonucleoprotein reverse transcriptase, referred to as "telomerase," which adds the characteristic telomere repeats (Greider *et al.*, *Cell*, 1985, 43, 405). Telomerase is not expressed in most somatic
20 cells and, as a consequence, these cells have a finite life-span. Extensive studies in a broad range of tumors indicate that 85-95% of all tumors cells express telomerase (see, e.g., Shay *et al.*, *Eur. J. Cancer*, 1997, 33, 787). Telomerase expression is an early event in tumor progression and is often present at the premalignant stage. Telomerase expression alone is not sufficient for tumorigenesis, but it is likely to be an essential permissive event
25 to allow the tumor to continue to grow.

 In addition to telomerase, another protein has been identified as capable of regulating telomere length, referred to as telomere repeat binding factor 1 (TRF1), that binds as a dimer to double stranded telomeric repeats (Chong *et al.*, *Science*, 1995, 270,

1663). Overexpression of TRF1 in a telomerase-expressing cell line leads to progressive telomere shortening, whereas inhibition of TRF1 increases telomere length (van Steensell *et al.*, *Nature*, 1997, 385, 740). TRF1 does not control the expression of telomerase itself but, rather, is thought to act by inhibiting telomerase action at the telomere termini (Smith *et al.*, *Science*, 1998, 282, 1484).

The ability of TRF1 to bind to telomeric DNA can be inhibited by its modification through the action of an enzyme called tankyrase, which covalently modifies TRF1 by ADP-ribosylation (Smith *et al.*, *Science*, 1998, 282, 1484). Tankyrase is a multidomain protein characterized by the presence of four distinct domains and are listed here in order from the polypeptide NH₂-terminus to the carboxy-terminus; HPS, ANK, SAM, and PARP. The HPS domain is a region containing homopolymeric runs of His, Pro, and Ser and has unknown function. The ANK domain contains 24 repeat units of a 33-amino acid ANK motif, as found in ankyrins. This domain is responsible for the binding of tankyrase to TRF1. The SAM domain (sterile alpha module) has unknown function but is believed to contribute to protein-protein interactions. The C-terminal catalytic active region is homologous to the active region of human Poly(ADP-Ribose) polymerase (PARP) enzymes. PARP catalyzes the formation of poly(ADP-ribose) onto a suitable protein receptor using NAD⁺ as substrate. The present invention involves the surprising discovery of a novel polypeptide, herein designated THP, that exhibits at least one functional homology to human tankyrase, and its role as a key component, for example, in regulating telomerase activity and thereby telomere length. THP is, thus, useful in the search for novel anti-tumor agents that can modify and/or control telomere length and/or telomere maintenance. These and other aspects of the invention are described below.

SUMMARY OF THE INVENTION

The present invention is directed to, in part, isolated nucleic acid molecules comprising SEQ ID NO:3, or a fragment thereof; SEQ ID NO:4, or a fragment thereof; a nucleotide sequence complementary to at least a portion of SEQ ID NO:3 or SEQ ID NO:4; a nucleotide sequence homologous to SEQ ID NO:3 or SEQ ID NO:4, or a fragment thereof; a nucleotide sequence that encodes a polypeptide comprising SEQ ID NO:5, or a fragment thereof; or a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence homologous to SEQ ID NO:5, or a fragment thereof.

The present invention is also directed to recombinant expression vectors comprising any of the nucleic acid molecules described above.

The present invention is also directed to host cells transformed with a recombinant expression vector comprising any of the nucleic acid molecules described above.

5 The present invention is also directed to methods of producing a polypeptide comprising SEQ ID NO:5, or a homolog or fragment thereof, by introducing a recombinant expression vector comprising any of the nucleic acid molecules described above into a compatible host cell, growing the host cell under conditions for expression of the polypeptide, and recovering the polypeptide from the host cell.

10 The present invention is also directed to compositions comprising any of the nucleic acid molecules described above and an acceptable carrier or diluent.

The present invention is also directed to isolated polypeptides encoded by any of the nucleic acid molecules described above.

15 The present invention is also directed to compositions comprising a polypeptide encoded by any of the nucleic acid molecules described above and an acceptable carrier or diluent.

The present invention is also directed to isolated antibodies which bind to an epitope on a polypeptide encoded by any of the nucleic acid molecules described above.

20 The present invention is also directed to kits comprising antibodies which bind to a polypeptide encoded by any of the nucleic acid molecules described above and a negative control antibody.

The present invention is also directed to methods of inducing an immune response in a mammal against a polypeptide encoded by any of the nucleic acid molecules described above by administering to the mammal an amount of the polypeptide sufficient to induce the immune response.

25 The present invention is also directed to methods of identifying a compound which binds to tankyrase homolog protein (THP) by contacting THP with a compound, and determining whether the compound binds THP.

30 The present invention is also directed to methods of identifying a compound which binds a nucleic acid molecule encoding tankyrase homolog protein (THP) by contacting THP with a compound, and determining whether the compound binds the nucleic acid molecule.

The present invention is also directed to methods of identifying a compound which modulates the activity of tankyrase homolog protein (THP) by contacting THP with a compound, and determining whether THP activity is modified.

35 The present invention is also directed to compounds which modulate THP activity identified by contacting THP with the compound, and determining whether the

compound modifies activity of THP, binds to THP, or binds to a nucleic acid molecule encoding THP.

These and other aspects of the invention are described in greater detail below.

5

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention provides, *inter alia*, isolated and purified polynucleotides that encode THP or a portion thereof, vectors containing these polynucleotides, host cells transformed with these vectors, processes of making THP, methods of using the above polynucleotides and vectors, isolated and purified THP, and methods of screening compounds which modulate THP activity.

Various definitions are made throughout this document. Most words have the meaning that would be attributed to those words by one skilled in the art. Words specifically defined either below or elsewhere in this document have the meaning provided in the context of the present invention as a whole and as are typically understood by those skilled in the art.

As used herein, the term "activity" refers to a variety of measurable indicia suggesting or revealing binding, either direct or indirect; affecting a response, *i.e.* having a measurable affect in response to some exposure or stimulus, including, for example, the affinity of a compound for directly binding a polypeptide or polynucleotide of the invention, or, for example, measurement of amounts of upstream or downstream proteins or other similar functions after some stimulus or event.

As used herein, the abbreviation in lower case (Thp) refers to a gene, cDNA, RNA or nucleic acid sequence while the upper case version (THP) refers to a protein, polypeptide, peptide, oligopeptide, or amino acid sequence.

As used herein, the term "antibody" is meant to refer to complete, intact antibodies, and Fab fragments and F(ab)₂ fragments thereof. Complete, intact antibodies include monoclonal antibodies such as murine monoclonal antibodies, chimeric antibodies and humanized antibodies.

As used herein, the term "binding" means the physical or chemical interaction between two proteins or compounds or associated proteins or compounds or combinations thereof. Binding includes ionic, non-ionic, Hydrogen bonds, Van der Waals, hydrophobic interactions, etc. The physical interaction, the binding, can be either direct or indirect, indirect being through or due to the effects of another protein or compound. Direct binding refers to interactions that do not take place through or due to the effect of another protein or compound but instead are without other substantial chemical intermediates.

As used herein, the term "compound" means any identifiable chemical or molecule, including, but not limited to, small molecule, peptide, protein, sugar, nucleotide, or nucleic acid, and such compound can be natural or synthetic.

As used herein, the term "complementary" refers to Watson-Crick basepairing between nucleotide units of a nucleic acid molecule.

As used herein, the term "contacting" means bringing together either directly or indirectly, a compound into physical proximity to a polypeptide or polynucleotide of the invention. The polypeptide or polynucleotide can be in any number of buffers, salts, solutions etc. Contacting includes, for example, placing the compound into a beaker, microtiter plate, cell culture flask, or a microarray, such as a gene chip, or the like, which contains the nucleic acid molecule or polypeptide encoding THP or a fragment thereof.

As used herein, the phrase "homologous nucleotide sequence," or "homologous amino acid sequence," or variations thereof, refers to sequences characterised by a homology, at the nucleotide level or amino acid level, of at least about 60%, more preferably at least about 70%, more preferably at least about 80%, more preferably at least about 90%, and most preferably at least about 95% to the entire SEQ ID NO:3 or SEQ ID NO:4, or to at least a portion of SEQ ID NO:3 or SEQ ID NO:4 which encodes a functional domain of the encoded polypeptide, or to SEQ ID NO:5. Homologous nucleotide sequences include those sequences coding for isoforms of THP proteins. Such isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. Homologous nucleotide sequences include nucleotide sequences encoding for a THP protein of species other than humans, including, but not limited to, mammals. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the nucleotide sequence encoding human tankyrase. Homologous amino acid sequences include those amino acid sequences which encode conservative amino acid substitutions in SEQ ID NO:5, as well as polypeptides having tankyrase activity. A homologous amino acid sequence does not, however, include the amino acid sequence encoding human tankyrase. Percent homology is preferably determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison WI), using the default settings, which uses the algorithm of Smith and Waterman (*Adv. Appl. Math.*, 1981, 2, 482-489, which is incorporated herein by reference in its entirety).

As used herein, the term "isolated" nucleic acid molecule refers to a nucleic acid molecule (DNA or RNA) that has been removed from its native environment. Examples of isolated nucleic acid molecules include, but are not limited to, recombinant DNA molecules contained in a vector, recombinant DNA molecules maintained in a heterologous host cell, partially or substantially purified nucleic acid molecules, and synthetic DNA or RNA molecules.

As used herein, the terms "modulates" or "modifies" means an increase or decrease in the amount, quality, or effect of a particular activity or protein.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues which has a sufficient number of bases to be used in a polymerase chain reaction (PCR). This short sequence is based on (or designed from) a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of a DNA sequence having at least about 10 nucleotides and as many as about 50 nucleotides, preferably about 15 to 30 nucleotides. They are chemically synthesized and may be used as probes.

As used herein, the term "probe" refers to nucleic acid sequences of variable length, preferably between at least about 10 and as many as about 6,000 nucleotides, depending on use. They are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are usually obtained from a natural or recombinant source, are highly specific and much slower to hybridize than oligomers. They may be single- or double-stranded and carefully designed to have specificity in PCR, hybridization membrane-based, or ELISA-like technologies.

As used herein, the phrase "stringent hybridization conditions" or "stringent conditions" refers to conditions under which a probe, primer, or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 5 C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present in excess, at T_m , 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30 C for short probes, primers or oligonucleotides (e.g. 10 to 50 nucleotides) and at least about 60 C for longer probes,

primers or oligonucleotides. Stringent conditions may also be achieved with the addition of destabilising agents, such as formamide:

The amino acid sequences are presented in the amino to carboxy direction, from left to right. The amino and carboxy groups are not presented in the sequence. The
5 nucleotide sequences are presented by single strand only, in the 5' to 3' direction, from left to right. Nucleotides and amino acids are represented in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by three letters code.

One aspect of the present invention is directed to nucleic acid molecules
10 comprising novel nucleotide sequences encoding THP. The nucleic acid molecules are preferably either RNA or DNA, but may contain both RNA and DNA monomers or peptide nucleic acid monomers. The nucleic acid molecule may be single stranded or double stranded. The monomers of the nucleic acid molecules may be linked via conventional phosphodiester bonds or modified bonds, such as, for example,
15 phosphorothioate bonds and the like. In addition, the sugar moieties of the monomers may be modified by, for example, addition of 2' substitutions which help confer nuclease resistance and/or cellular uptake.

In a preferred embodiment of the invention, the nucleic acid molecule comprises SEQ ID NO:3, which is 4512 bases in length and comprises an open reading
20 frame (ORF) of approximately 3498 nucleotides (from about position 23 to about position 3520 within SEQ ID NO:3) which encodes human THP. Alternatively, the nucleic acid molecule comprises a fragment of SEQ ID NO:3. Preferably, the fragment comprises from about 10 to about 100 nucleotides, from about 101 to about 200 nucleotides, from about 201 to about 300 nucleotides, from about 301 to about 400 nucleotides, from about
25 401 to about 500 nucleotides, from about 501 to about 600 nucleotides, from about 601 to about 700 nucleotides, from about 701 to about 800 nucleotides, from about 801 to about 900 nucleotides, from about 901 to about 1000 nucleotides, from about 1001 to about 1100 nucleotides, from about 1101 to about 1200 nucleotides, from about 1201 to about 1300 nucleotides, from about 1301 to about 1400 nucleotides, from about 1401 to about 1500
30 nucleotides, from about 1501 to about 1600 nucleotides, from about 1601 to about 1700 nucleotides, from about 1701 to about 1800 nucleotides, from about 1801 to about 1900 nucleotides, from about 1901 to about 2000 nucleotides, from about 2001 to about 2100 nucleotides, from about 2101 to about 2200 nucleotides, from about 2201 to about 2300 nucleotides, from about 2301 to about 2400 nucleotides, from about 2401 to about 2500
35 nucleotides, from about 2501 to about 2600 nucleotides, from about 2601 to about 2700 nucleotides, from about 2701 to about 2800 nucleotides, from about 2801 to about 2900 nucleotides, from about 2901 to about 3000 nucleotides, from about 3001 to about 3100

nucleotides, from about 3101 to about 3200 nucleotides, from about 3201 to about 3300 nucleotides, from about 3301 to about 3400 nucleotides, from about 3401 to about 3500 nucleotides, from about 3501 to about 3600 nucleotides, from about 3601 to about 3700 nucleotides, from about 3701 to about 3800 nucleotides, from about 3801 to about 3900 nucleotides, from about 3901 to about 4000 nucleotides, from about 4001 to about 4100 nucleotides, from about 4101 to about 4200 nucleotides, from about 4201 to about 4300 nucleotides, from about 4301 to about 4400 nucleotides, from about 4401 to about 4500 nucleotides, or from about 4501 to about 4511 nucleotides, and any combinations thereof. The fragment can be located within any portion of SEQ ID NO:3.

10 In another preferred embodiment of the invention, the nucleic acid molecule comprises SEQ ID NO:4, which is 3498 bases in length and comprises the ORF (from about position 23 to about position 3520 within SEQ ID NO:3) described above. Alternatively, the nucleic acid molecule comprises a fragment of SEQ ID NO:4. Preferably, the fragment comprises from about 10 to about 100 nucleotides, from about 101 to about 200 nucleotides, from about 201 to about 300 nucleotides, from about 301 to about 400 nucleotides, from about 401 to about 500 nucleotides, from about 501 to about 600 nucleotides, from about 601 to about 700 nucleotides, from about 701 to about 800 nucleotides, from about 801 to about 900 nucleotides, from about 901 to about 1000 nucleotides, from about 1001 to about 1100 nucleotides, from about 1101 to about 1200 nucleotides, from about 1201 to about 1300 nucleotides, from about 1301 to about 1400 nucleotides, from about 1401 to about 1500 nucleotides, from about 1501 to about 1600 nucleotides, from about 1601 to about 1700 nucleotides, from about 1701 to about 1800 nucleotides, from about 1801 to about 1900 nucleotides, from about 1901 to about 2000 nucleotides, from about 2001 to about 2100 nucleotides, from about 2101 to about 2200 nucleotides, from about 2201 to about 2300 nucleotides, from about 2301 to about 2400 nucleotides, from about 2401 to about 2500 nucleotides, from about 2501 to about 2600 nucleotides, from about 2601 to about 2700 nucleotides, from about 2701 to about 2800 nucleotides, from about 2801 to about 2900 nucleotides, from about 2901 to about 3000 nucleotides, from about 3001 to about 3100 nucleotides, from about 3101 to about 3200 nucleotides, from about 3201 to about 3300 nucleotides, from about 3301 to about 3400 nucleotides, or from about 3401 to about 3497 nucleotides, and any combinations thereof. The fragment can be located within any portion of SEQ ID NO:4.

In another preferred embodiment of the invention, the nucleic acid molecule comprises a nucleotide sequence complementary to at least a portion of SEQ ID NO:3 or SEQ ID NO:4. Preferably, the nucleic acid molecule comprises a nucleotide sequence complementary to the entire sequence recited in SEQ ID NO:3 or SEQ ID NO:4. Alternatively, the nucleic acid molecule comprises a nucleotide sequence complementary

to a portion of SEQ ID NO:3 or SEQ ID NO:4 (i.e., complementary to any of the fragments described above). Nucleotide sequences complementary to at least a portion of SEQ ID NO:3 or SEQ ID NO:4 include, for example, oligonucleotides which hybridize under stringent hybridization conditions to at least a portion of SEQ ID NO:3 or SEQ ID NO:4. Preferred oligonucleotides comprise at least about 10 nucleotides and as many as about 50 nucleotides, preferably about 15 to 30 nucleotides. They are chemically synthesized and can be used as probes, primers, and as antisense agents.

In another preferred embodiment of the invention, the nucleic acid molecule comprises a nucleotide sequence homologous to SEQ ID NO:3 or SEQ ID NO:4. Preferably, the nucleotide sequence is at least about 75% homologous, more preferably at least about 80% homologous, more preferably at least about 90% homologous, and most preferably at least about 95% homologous to the entire SEQ ID NO:3 or SEQ ID NO:4. Alternatively, the nucleotide sequence is at least about 60% homologous, more preferably at least about 70% homologous, more preferably at least about 80% homologous, more preferably at least about 90% homologous, and most preferably at least about 95% homologous to a portion of SEQ ID NO:3 or SEQ ID NO:4 which encodes a functional domain of the polypeptide encoded thereby. In addition, a nucleotide sequence homologous to SEQ ID NO:3 or SEQ ID NO:4 also includes a fragment of the nucleotide sequence homologous to SEQ ID NO:3 or SEQ ID NO:4 of the lengths described above.

In another preferred embodiment of the invention, the nucleic acid molecule comprises a nucleotide sequence that encodes a polypeptide comprising SEQ ID NO:5. The nucleic acid molecule preferably comprises SEQ ID NO:4 or comprises SEQ ID NO:4 containing codon substitutions which reflect the degeneracy of the genetic code. As is well known in the art, because of the degeneracy of the genetic code, there are numerous other DNA and RNA molecules that can code for the same polypeptide as that encoded by SEQ ID NO:4. The present invention, therefore, contemplates these other DNA and RNA molecules which, on expression, encode the polypeptide of SEQ ID NO:5. DNA and RNA molecules other than those specifically disclosed herein characterised simply by a change in a codon for a particular amino acid, are within the scope of the present invention.

As is well known in the art, because of the degeneracy of the genetic code, there are numerous other DNA and RNA molecules that can code for the same polypeptide as that encoded by the aforementioned THP gene. The present invention, therefore, contemplates those other DNA and RNA molecules which, on expression, encode the polypeptide of SEQ ID NO: 4. Having identified the amino acid residue sequence encoded by a Thp gene, and with knowledge of all triplet codons for each particular amino acid residue, it is possible to describe all such encoding RNA and DNA sequences, DNA and

RNA molecules other than those specifically disclosed herein characterised simply by a change in a codon for a particular amino acid, are within the scope of this invention.

A table of amino acids and their representative abbreviations, symbols and codons is set forth below in the following Table 1.

5

Table 1						
Amino acid	Abbrev.	Symbol	Codon(s)			
Alanine	Ala	A	GCA	GCC	GCG	GCU
Cysteine	Cys	C	UGA	UGU		
Aspartic acid	Asp	D	GAC	GAU		
Glutamic acid	Glu	E	GAA	GAG		
Phenylalanine	Phe	F	UUC	UUU		
Glycine	Gly	G	GGA	GGC	GGG	GGU
Histidine	His	H	CAC	CAU		
Isoleucine	Ile	I	AUA	AUC	AUU	
Lysine	Lys	K	AAA	AAG		
Leucine	Leu	L	UUA	UUG	CUA	CUC
Methionine	Met	M	AUG			
Asparagine	Asn	N	AAC	AAU		
Proline	Pro	P	CCA	CCC	CCG	CCU
Glutamine	Gln	Q	CAA	CAG		
Arginine	Arg	R	AGA	AGG	CGA	CGC
Serine	Ser	S	AGC	AGU	UCA	UCC
Threonine	Thr	T	ACA	ACC	ACG	ACU
Valine	Val	V	GUA	GUC	GUG	GUU
Tryptophan	Trp	W	UGG			
Tyrosine	Tyr	Y	UAC	UAU		

As is well known in the art, codons constitute triplet sequences of nucleotides in mRNA molecules and, as such, are characterised by the base uracil (U) in place of base thymidine (T) (which is present in DNA molecules). A simple change in a codon for the same amino acid residue within a polynucleotide will not change the sequence or structure of the encoded polypeptide.

Alternatively, the nucleic acid molecule comprises a nucleotide sequence that encodes a fragment of the polypeptide encoding SEQ ID NO:5. Preferably, the fragment comprises from about 5 to about 20 amino acids, from about 21 to about 40 amino acids, from about 41 to about 60 amino acids, from about 61 to about 80 amino acids, from about 81 to about 100 amino acids, from about 101 to about 120 amino acids, from about 121 to about 140 amino acids, from about 141 to about 160 amino acids, from about 161 to about 180 amino acids, from about 181 to about 200 amino acids, from about 201 to about 220 amino acids, from about 221 to about 240 amino acids, from about 241 to about 260 amino acids, from about 261 to about 280 amino acids, from about 281 to about 300 amino acids, from about 301 to about 320 amino acids, from about 321 to about 340

amino acids, from about 341 to about 360 amino acids, from about 361 to about 380 amino acids, from about 381 to about 400 amino acids, from about 401 to about 420 amino acids, from about 421 to about 440 amino acids, from about 441 to about 460 amino acids, from about 461 to about 480 amino acids, from about 481 to about 500 amino acids, from about 501 to about 520 amino acids, from about 521 to about 540 amino acids, from about 541 to about 560 amino acids, from about 561 to about 580 amino acids, from about 581 to about 600 amino acids, ~~from about 601 to about 620 amino acids, from about 621 to about 640~~ amino acids, from about 641 to about 660 amino acids, from about 661 to about 680 amino acids, from about 681 to about 700 amino acids, from about 701 to about 720 amino acids, from about 721 to about 740 amino acids, from about 741 to about 760 amino acids, from about 761 to about 780 amino acids, from about 781 to about 800 amino acids, from about 801 to about 820 amino acids, from about 821 to about 840 amino acids, from about 841 to about 860 amino acids, from about 861 to about 880 amino acids, from about 881 to about 900 amino acids, from about 901 to about 920 amino acids, from about 921 to about 940 amino acids, from about 941 to about 960 amino acids, from about 961 to about 980 amino acids, from about 981 to about 1000 amino acids, from about 1001 to about 1020 amino acids, from about 1021 to about 1040 amino acids, from about 1041 to about 1060 amino acids, from about 1061 to about 1080 amino acids, from about 1081 to about 1100 amino acids, from about 1101 to about 1120 amino acids, from about 1121 to about 1140 amino acids, from about 1141 to about 1160 amino acids, or from about 1161 to about 1165 amino acids, and any combinations thereof. The fragment can be located within any portion of SEQ ID NO:5.

In another preferred embodiment of the invention, the nucleic acid molecule comprises a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence homologous to SEQ ID NO:5. Alternatively, the nucleic acid molecule comprises a nucleotide sequence that encodes a fragment of the polypeptide comprising an amino acid sequence homologous to SEQ ID NO:5. Preferably, the fragment comprises from about 5 to about 20 amino acids, from about 21 to about 40 amino acids, from about 41 to about 60 amino acids, from about 61 to about 80 amino acids, from about 81 to about 100 amino acids, from about 101 to about 120 amino acids, from about 121 to about 140 amino acids, from about 141 to about 160 amino acids, from about 161 to about 180 amino acids, from about 181 to about 200 amino acids, from about 201 to about 220 amino acids, from about 221 to about 240 amino acids, from about 241 to about 260 amino acids, from about 261 to about 280 amino acids, from about 281 to about 300 amino acids, from about 301 to about 320 amino acids, from about 321 to about 340 amino acids, from about 341 to about 360 amino acids, from about 361 to about 380 amino acids, from about 381 to about 400 amino acids, from about 401 to about 420 amino acids,

from about 421 to about 440 amino acids, from about 441 to about 460 amino acids, from about 461 to about 480 amino acids, from about 481 to about 500 amino acids, from about 501 to about 520 amino acids, from about 521 to about 540 amino acids, from about 541 to about 560 amino acids, from about 561 to about 580 amino acids, from about 581 to about 5 600 amino acids, from about 601 to about 620 amino acids, from about 621 to about 640 amino acids, from about 641 to about 660 amino acids, from about 661 to about 680 amino acids, from about 681 to about 700 amino acids, from about 701 to about 720 amino acids, from about 721 to about 740 amino acids, from about 741 to about 760 amino acids, from about 761 to about 780 amino acids, from about 781 to about 800 amino acids, from about 801 to about 820 amino acids, from about 821 to about 840 amino acids, from about 841 to about 860 amino acids, from about 861 to about 880 amino acids, from about 881 to about 900 amino acids, from about 901 to about 920 amino acids, from about 921 to about 940 amino acids, from about 941 to about 960 amino acids, from about 961 to about 980 amino acids, from about 981 to about 1000 amino acids, from about 1001 to about 1020 amino acids, from about 1021 to about 1040 amino acids, from about 1041 to about 1060 amino acids, from about 1061 to about 1080 amino acids, from about 1081 to about 1100 amino acids, from about 1101 to about 1120 amino acids, from about 1121 to about 1140 amino acids, from about 1141 to about 1160 amino acids, or from about 1161 to about 1165 amino acids, and any combinations thereof. The fragment can be located within any 20 portion of SEQ ID NO:5.

With the knowledge of the nucleotide sequence information disclosed in the present invention, one skilled in the art can identify and obtain nucleotide sequences which encode THP from different sources (*i.e.*, different tissues or different organisms) through a variety of means well known to the skilled artisan and disclosed by, for example, 25 Sambrook et al., "Molecular cloning: a laboratory manual", Second Edition, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), which is incorporated herein by reference in its entirety.

For example, DNA which encodes the THP may be obtained by screening of mRNA, cDNA, or genomic DNA with oligonucleotide probes generated from the Thp gene sequence information provided herein. Probes may be labeled with a detectable 30 group, such as a fluorescent group, a radioactive atom or a chemiluminescent group in accordance with procedures known to the skilled artisan and used in conventional hybridization assays, as described by, for example, Sambrook et al.

A nucleic acid molecule comprising any of the Thp nucleotide sequences 35 described above can alternatively be recovered by use of the polymerase chain reaction (PCR) procedure, with the PCR oligonucleotide primers produced from the nucleotide sequences provided herein. See U.S. Patent Numbers 4,683,195 to Mullis *et al.* and

4,683,202 to Mullis. The PCR reaction provides a method for selectively increasing the concentration of a particular nucleic acid sequence even when that sequence has not been previously purified and is present only in a single copy in a particular sample. The method can be used to amplify either single- or double-stranded DNA. The essence of the method involves the use of two oligonucleotides probes to serve as primers for the template-dependent, polymerase mediated replication of a desired nucleic acid molecule.

A wide variety of alternative cloning and *in vitro* amplification methodologies are well known to those skilled in the art. Examples of these techniques are found in, for example, Berger *et al.*, *Guide to Molecular Cloning Techniques*, Methods in Enzymology 152 Academic Press, Inc., San Diego, CA (Berger), which is incorporated herein by reference in its entirety.

The nucleic acid molecules of the present invention, and fragments derived therefrom, are useful for screening for restriction fragment length polymorphism (RFLP) associated with certain disorders, as well as for genetic mapping.

Antisense oligonucleotides, or fragments of SEQ ID NO:3 or SEQ ID NO:4, or sequences complementary thereto, derived from the nucleotide sequences of the present invention encoding THP are useful as diagnostic tools for probing Thp gene expression in various tissues. For example, tissue can be probed *in situ* with oligonucleotide probes carrying detectable groups by conventional autoradiography techniques to investigate native expression of this enzyme or pathological conditions relating thereto. Antisense oligonucleotides are preferably directed to regulatory regions of SEQ ID NO:3 or SEQ ID NO:4 or mRNA corresponding thereto, including, but not limited to, the initiation codon, TATA box, enhancer sequences, and the like.

Automated sequencing methods were used to obtain or verify the nucleotide sequence of THP. The THP nucleotide sequences of the present invention were obtained for both DNA strands, and are believed to be 100% accurate. However, as is known in the art, nucleotide sequence obtained by automated methods may contain some errors. Nucleotide sequences determined by automation are typically at least about 90%, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of a given nucleic acid molecule. The actual sequence may be more precisely determined using manual sequencing methods, which are well known in the art. An error in sequence which results in an insertion or deletion of one or more nucleotides may result in a frame shift in translation such that the predicted amino acid sequence will differ from that which would be predicted from the actual nucleotide sequence of the nucleic acid molecule, starting at the point of the mutation.

Another aspect of the present invention is directed to vectors, or recombinant expression vectors, comprising any of the nucleic acid molecules described

above. Vectors are used herein either to amplify DNA or RNA encoding THP and/or to express DNA which encodes THP. Preferred vectors include, but are not limited to, plasmids, phages, cosmids, viral particles or viruses, and integratable DNA fragments (*i.e.*, fragments integratable into the host genome by homologous recombination). Preferred
5 viral particles include, but are not limited to, adenoviruses, parvoviruses, herpesviruses, poxviruses, adeno-associated viruses, Semliki Forest viruses, vaccinia viruses, and retroviruses. Preferred expression vectors include, but are not limited to, pcDNA3 (Invitrogen) and pSVL (Pharmacia Biotech). Other expression vectors include, but are not limited to, pSPORT vectors, pGEM vectors (Promega), pPROEXvectors (LTI, Bethesda,
10 MD), Bluescript vectors (Stratagene), pQE vectors (Qiagen), pSE420 (Invitrogen), and pYES2 (Invitrogen).

Preferred expression vectors are replicable DNA constructs in which a DNA sequence encoding THP is operably linked to suitable control sequences capable of effecting the expression of the THP in a suitable host. DNA regions are operably linked
15 when they are functionally related to each other. For example, a promoter is operably linked to a coding sequence if it controls the transcription of the sequence. Amplification vectors do not require expression control domains, but rather need only the ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants. The need for control sequences into the expression
20 vector will vary depending upon the host selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding, and sequences which control the termination of transcription and translation.

Preferred vectors preferably contain a promoter which is recognised by the
25 host organism. The promoter sequences of the present invention may be either prokaryotic, eukaryotic or viral. Examples of suitable prokaryotic sequences include the P_R and P_L promoters of bacteriophage lambda (The bacteriophage Lambda, Hershey, A. D., Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (1973), which is incorporated herein by reference in its entirety; Lambda II, Hendrix, R. W., Ed., Cold Spring Harbor
30 Press, Cold Spring Harbor, NY (1980), which is incorporated herein by reference in its entirety); the trp, recA, heat shock, and lacZ promoters of *E. coli* and the SV40 early promoter (Benoist, *et al. Nature*, 1981, 290, 304-310, which is incorporated herein by reference in its entirety). Additional promoters include, but are not limited to, mouse mammary tumor virus, long terminal repeat of human immunodeficiency virus, maloney
35 virus, cytomegalovirus immediate early promoter, Epstein Barr virus, rous sarcoma virus, human actin, human myosin, human hemoglobin, human muscle creatine, and human metallothionein.

Additional regulatory sequences can also be included in preferred vectors. Preferred examples of suitable regulatory sequences are represented by the Shine-Dalgarno of the replicase gene of the phage MS-2 and of the gene cII of bacteriophage lambda. The Shine-Dalgarno sequence may be directly followed by the DNA encoding THP and result in the expression of the mature THP protein.

Moreover, suitable expression vectors can include an appropriate marker which allows the screening of the transformed host cells. The transformation of the selected host is carried out using any one of the various techniques well known to the expert in the art and described in Sambrook et al., *supra*.

An origin of replication can also be provided either by construction of the vector to include an exogenous origin or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter may be sufficient. Alternatively, rather than using vectors which contains viral origins of replication, one skilled in the art can transform mammalian cells by the method of co-transformation with a selectable marker and THP DNA. An example of a suitable marker is dihydrofolate reductase (DHFR) or thymidine kinase (*see*, U.S. Patent No. 4,399,216).

Nucleotide sequences encoding THP may be recombined with vector DNA in accordance with conventional techniques, including blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulation are disclosed by Sambrook et al., *supra* and are well known in the art. Methods for construction of mammalian expression vectors are disclosed in, for example, Okayama et al., *Mol. Cell. Biol.*, 1983, 3, 280, Cosman et al., *Mol. Immunol.*, 1986, 23, 935, Cosman et al., *Nature*, 1984, 312, 768, EP-A-0367566, and WO 91/18982, each of which is incorporated herein by reference in its entirety.

Another aspect of the present invention is directed to transformed host cells having an expression vector comprising any of the nucleic acid molecules described above. Expression of the nucleotide sequence occurs when the expression vector is introduced into an appropriate host cell. Suitable host cells for expression of the polypeptides of the invention include, but are not limited to, prokaryotes, yeast, and eukaryotes. If a prokaryotic expression vector is employed, then the appropriate host cell would be any prokaryotic cell capable of expressing the cloned sequences. Suitable prokaryotic cells include, but are not limited to, bacteria of the genera *Escherichia*, *Bacillus*, *Salmonella*, *Pseudomonas*, *Streptomyces*, and *Staphylococcus*.

If a eukaryotic expression vector is employed, then the appropriate host cell would be any eukaryotic cell capable of expressing the cloned sequence. Preferably,

eukaryotic cells are cells of higher eukaryotes. Suitable eukaryotic cells include, but are not limited to, non-human mammalian tissue culture cells and human tissue culture cells. Preferred host cells include, but are not limited to, insect cells, HeLa cells, Chinese hamster ovary cells (CHO cells), African green monkey kidney cells (COS cells), human 293 cells, and murine 3T3 fibroblasts. Propagation of such cells in cell culture has become a routine procedure (*see*, Tissue Culture, Academic Press, Kruse and Patterson, eds. (1973), which is incorporated herein by reference in its entirety).

In addition, a yeast host may be employed as a host cell. Preferred yeast cells include, but are not limited to, the genera *Saccharomyces*, *Pichia*, and *Kluveromyces*. Preferred yeast hosts are *S. cerevisiae* and *P. pastoris*. Preferred yeast vectors can contain an origin of replication sequence from a 2T yeast plasmid, an autonomously replication sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination, and a selectable marker gene. Shuttle vectors for replication in both yeast and *E. coli* are also included herein.

Alternatively, insect cells may be used as host cells. In a preferred embodiment, the polypeptides of the invention are expressed using a baculovirus expression system (*see*, Luckow *et al.*, *Bio/Technology*, 1988, 6, 47, Baculovirus Expression Vectors: A Laboratory Manual, O'Rielly *et al.* (Eds.), W.H. Freeman and Company, New York, 1992, and U.S. Patent No. 4,879,236, each of which is incorporated herein by reference in its entirety). In addition, the MAXBAC™ complete baculovirus expression system (Invitrogen) can, for example, be used for production in insect cells.

Another aspect of the present invention is directed to compositions, including pharmaceutical compositions, comprising any of the nucleic acid molecules or recombinant expression vectors described above and an acceptable carrier or diluent. Preferably, the carrier or diluent is pharmaceutically acceptable. Suitable carriers are described in the most recent edition of *Remington's Pharmaceutical Sciences*, A. Osol, a standard reference text in this field, which is incorporated herein by reference in its entirety. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils may also be used. The formulations are sterilized by commonly used techniques.

Another aspect of the present invention is directed to an isolated polypeptide encoded by a nucleic acid molecule described above. In preferred embodiments of the invention, the isolated polypeptide comprises the amino acid sequence set forth in SEQ ID NO:5. Alternatively, the polypeptide is a fragment of the polypeptide encoding SEQ ID NO:5. Preferably, the fragment comprises from about 5 to about 20 amino acids, from about 21 to about 40 amino acids, from about 41 to about 60 amino

acids, from about 61 to about 80 amino acids, from about 81 to about 100 amino acids, from about 101 to about 120 amino acids, from about 121 to about 140 amino acids, from about 141 to about 160 amino acids, from about 161 to about 180 amino acids, from about 181 to about 200 amino acids, from about 201 to about 220 amino acids, from about 221 to about 240 amino acids, from about 241 to about 260 amino acids, from about 261 to about 280 amino acids, from about 281 to about 300 amino acids, from about 301 to about 320 amino acids, ~~from about 321 to about 340 amino acids, from about 341 to about 360 amino acids, from about 361 to about 380 amino acids, from about 381 to about 400 amino acids, from about 401 to about 420 amino acids, from about 421 to about 440 amino acids, from about 441 to about 460 amino acids, from about 461 to about 480 amino acids, from about 481 to about 500 amino acids, from about 501 to about 520 amino acids, from about 521 to about 540 amino acids, from about 541 to about 560 amino acids, from about 561 to about 580 amino acids, from about 581 to about 600 amino acids, from about 601 to about 620 amino acids, from about 621 to about 640 amino acids, from about 641 to about 660 amino acids, from about 661 to about 680 amino acids, from about 681 to about 700 amino acids, from about 701 to about 720 amino acids, from about 721 to about 740 amino acids, from about 741 to about 760 amino acids, from about 761 to about 780 amino acids, from about 781 to about 800 amino acids, from about 801 to about 820 amino acids, from about 821 to about 840 amino acids, from about 841 to about 860 amino acids, from about 861 to about 880 amino acids, from about 881 to about 900 amino acids, from about 901 to about 920 amino acids, from about 921 to about 940 amino acids, from about 941 to about 960 amino acids, from about 961 to about 980 amino acids, from about 981 to about 1000 amino acids, from about 1001 to about 1020 amino acids, from about 1021 to about 1040 amino acids, from about 1041 to about 1060 amino acids, from about 1061 to about 1080 amino acids, from about 1081 to about 1100 amino acids, from about 1101 to about 1120 amino acids, from about 1121 to about 1140 amino acids, from about 1141 to about 1160 amino acids, or from about 1161 to about 1165 amino acids, and any combinations thereof. The fragment can be located within any portion of SEQ ID NO:5.~~

In another preferred embodiment of the invention, the polypeptide comprises an amino acid sequence homologous to SEQ ID NO:5 or a fragment thereof as described above. It is to be understood that the present invention includes proteins homologous to, and having essentially at least one biological property that is substantially similar to a biological property of the polypeptide. Preferably, the extent of the activity of the biological property is at least 10%, more preferably at least 20%, more preferably at least 30%, more preferably at least 40%, more preferably at least 50%, more preferably at least 60%, more preferably at least 70%, more preferably at least 80%, more preferably at least 90%, and most preferably 100% of the activity of the biological property of THP

(e.g., SEQ ID NO:5). Such proteins are also called variants. This definition is intended to encompass isoforms, natural allelic variants, and splice variants of the Thp gene described herein. These variant forms may result from, for example, alternative splicing or differential expression in different tissue of the same source organism. The variant forms may be characterised by, for example, amino acid insertion(s), deletion(s) or substitution(s). In this connection, a variant form having an amino acid sequence which has at least about 70% sequence homology, at least about 80% sequence homology, preferably about 90% sequence homology, more preferably about 95% sequence homology and most preferably about 98% sequence homology to SEQ ID NO:5, is contemplated as being included in the present invention. A preferred homologous polypeptide comprises at least one conservative amino acid substitution compared to SEQ ID NO:5. Amino acid "insertions", "substitutions" or "deletions" are changes to or within an amino acid sequence. The variation allowed in a particular amino acid sequence may be experimentally determined by producing the peptide synthetically or by systematically making insertions, deletions, or substitutions of nucleotides in the Thp sequence using recombinant DNA techniques.

Alterations of the naturally occurring amino acid sequence can be accomplished by any of a number of known techniques. For example, mutations can be introduced into the polynucleotide encoding a polypeptide at particular locations by procedures well known to the skilled artisan, such as oligonucleotide-directed mutagenesis, which is described by Walder *et al.*, *Gene*, 1986, 42, 133, Bauer *et al.*, *Gene*, 1985, 37, 73, Craik, *BioTechniques*, January 1985, pp.12-19, Smith *et al.*, *Genetic Engineering: Principles and Methods*, Plenum Press (1981), and U.S. Patent Numbers 4,518,584 and 4,737,462, each of which is incorporated herein by reference in its entirety.

Preferably, a THP variant of the present invention will exhibit substantially the biological activity of a naturally occurring THP polypeptide. By "exhibit substantially the biological activity of a naturally occurring THP polypeptide" is meant that THP variants within the scope of the invention can comprise conservatively substituted sequences, meaning that one or more amino acid residues of a THP polypeptide are replaced by different residues that do not alter the secondary and/or tertiary structure of the THP polypeptide. Such substitutions may include the replacement of an amino acid by a residue having similar physicochemical properties, such as substituting one aliphatic residue (Ile, Val, Leu or Ala) for another, or substitution between basic residues Lys and Arg, acidic residues Glu and Asp, amide residues Gln and Asn, hydroxyl residues Ser and Tyr, or aromatic residues Phe and Tyr. Further information regarding making phenotypically silent amino acid exchanges can be found in Bowie *et al.*, *Science*, 1990, 247, 1306-1310, which is incorporated herein by reference in its entirety. Other THP

variants which might retain substantially the biological activities of THP are those where amino acid substitutions have been made in areas outside functional regions of the protein.

The polypeptides to be expressed in such host cells may also be fusion proteins which include regions from heterologous proteins. Such regions may be included to allow, *e.g.*, secretion, improved stability, or facilitated purification of the polypeptide. For example, a sequence encoding an appropriate signal peptide can be incorporated into expression vectors. ~~A DNA sequence for a signal peptide (secretory leader) may be fused~~ in-frame to the polynucleotide sequence so that the polypeptide is translated as a fusion protein comprising the signal peptide. A signal peptide that is functional in the intended host cell promotes extracellular secretion of the polypeptide. Preferably, the signal sequence will be cleaved from the polypeptide upon secretion of the polypeptide from the cell. Thus, preferred fusion proteins can be produced in which the N-terminus of THP is fused to a carrier peptide.

In one embodiment, the polypeptide comprises a fusion protein which includes a heterologous region used to facilitate purification of the polypeptide. Many of the available peptides used for such a function allow selective binding of the fusion protein to a binding partner. A preferred binding partner includes one or more of the IgG binding domains of protein A are easily purified to homogeneity by affinity chromatography on, for example, IgG-coupled Sepharose. Alternatively, many vectors have the advantage of carrying a stretch of histidine residues that can be expressed at the N-terminal or C-terminal end of the target protein. Thus the protein of interest can be recovered by metal chelation chromatography. A nucleotide sequence encoding a recognition site for a proteolytic enzyme such as enterokinase, factor X or, procollagenase or thrombin may immediately precede the sequence for THP to permit cleavage of the fusion protein to obtain the mature THP protein. Additional examples of fusion partners include, but are not limited to, the yeast I-factor, the honeybee melatin leader in sf9 insect cells, 6-His tag, thioredoxin tag, hemagglutinin tag, GST tag, and OmpA signal sequence tag. As will be understood by one of skill in the art, the binding partner which recognizes and binds to the peptide may be any molecule or compound including metal ions (*e.g.*, metal affinity columns), antibodies, or fragments thereof, and any protein or peptide which binds the peptide, such as the FLAG tag.

The polypeptides of the invention can be used as antigens for raising antibodies against the same and used to screen for compounds that modulate the activity of THP. THP can also be used in compositions. Accordingly, the invention relates to THP or an antibody according to the invention for use as a medicament as well as to the use of the molecules in the manufacture of a medicament directed towards cancers or tumors, such as, for example, basal cell carcinomas. The molecule used as medicaments according

to the invention may be the polypeptides or antibodies described herein as well as any novel substance identified in a screening method described herein.

In another aspect, the invention provides THP polypeptides with or without associated native pattern glycosylation, acylation, sialylation, or other post-translational
5 modifications. THP expressed in yeast or mammalian expression systems (discussed below) may be similar to or significantly different from a native THP polypeptide in molecular weight and glycosylation pattern. Of course, expression of THP in bacterial expression systems will provide non-glycosylated THP.

Another aspect of the present invention is directed to compositions,
10 including pharmaceutical compositions, comprising any of the polypeptides described above and an acceptable carrier or diluent. Preferably, the carrier or diluent is pharmaceutically acceptable. Compositions comprising a polypeptide, as described above, can be used to, for example, induce antibody formation and to induce an immune response for use in, for example, vaccine preparations.

Another aspect of the present invention is directed to methods of producing
15 a polypeptide comprising SEQ ID NO:5, or a homolog or fragment thereof, comprising introducing any of the recombinant expression vectors described above into compatible host cells, growing the host cells under conditions for expression of the polypeptide, and recovering the polypeptide from the host cells. Eukaryotic systems are preferred since
20 they provide a variety of processing mechanisms which result in, for example, glycosylation, carboxy-terminal amidation, oxidation or derivatization of certain amino acid residues, conformational control, and so forth.

The polypeptides of the present invention are preferably provided in an isolated form, are preferably substantially purified, and most preferably are purified to
25 homogeneity. Host cells are preferably lysed and the polypeptide is recovered from the lysate of the host cells. Alternatively, the polypeptide is recovered by purifying the cell culture medium from the host cells, preferably without lysing the host cell. The polypeptides can be recovered and purified from recombinant cell cultures by well-known methods, including ammonium sulfate or ethanol precipitation, anion or cation exchange
30 chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography.

In addition to producing these proteins by recombinant techniques, automated amino acid synthesizers may also be employed to produce THP polypeptides,
35 or fragments a homologous protein thereof.

Another aspect of the present invention is directed to an antibody or antibodies which bind to an epitope on any of the polypeptides described herein.

Preferably, the antibody binds to an epitope within SEQ ID NO:5. The antibodies according to the invention can be monoclonal or polyclonal and include individual, allelic, strain or species variants, or fragments thereof, both in their naturally occurring (full-length) forms and recombinant forms. Additionally, the antibodies are raised to the present proteins in either their native configuration or in non-native configurations. Anti-idiotypic antibodies can also be generated. Hybridomas which produce antibodies that bind to the polypeptides of the invention, and the antibodies themselves, are useful in the isolation and purification of the polypeptides. In addition, antibodies may be specific inhibitors of THP activity. Antibodies which specifically bind to the polypeptides of the invention can be used to purify the protein from natural sources using well known techniques and readily available starting materials. Such antibodies can also be used to purify the protein from material present when producing the protein by recombinant DNA methodology.

Many methods of making antibodies are known to persons skilled in the art. For techniques for preparing monoclonal antibodies, see e.g. Stites et al (eds.), *Basic and Clinical Immunology* (4th ed), Lange Medical Publications, Los Altos, CA, which is incorporated herein by reference in its entirety, and references cited therein. Techniques that involve selection of libraries of recombinant antibodies in phage or similar vectors are described in Huse et al., *Science*, 1989, 246, 1275-1281, which is incorporated herein by reference in its entirety. The production of antibodies and the protein structures of complete, intact antibodies, Fab fragments and F(ab)₂ fragments and the organization of the genetic sequences that encode such molecules are well known and are also described, for example, in Harlow, E. and D. Lane (1988) *ANTIBODIES: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, which is incorporated herein by reference. Briefly, for example, a polypeptide of the invention is injected into mice. The spleen of the mouse is removed, the spleen cells are isolated and fused with immortalized mouse cells. The hybrid cells, or hybridomas, are cultured and those cells which secrete antibodies are selected. The antibodies are analyzed and, if found to specifically bind to the polypeptide, the hybridoma which produces them is cultured to produce a continuous supply of antibodies.

The present invention is also directed to kits, including pharmaceutical kits. The kits can comprise any of the THP nucleic acid molecules described above, any of the THP polypeptides described above, or any anti-THP antibody which binds to a polypeptide of the invention as described above, as well as a negative control. The kit preferably comprises additional components, such as, for example, instructions, solid support, reagents helpful for quantification, and the like.

Another aspect of the present invention is directed to methods of inducing an immune response in a mammal against a polypeptide of the invention by administering

to the mammal an amount of the polypeptide sufficient to induce an immune response. The amount will be dependent on the animal species, size of the animal, and the like but can be determined by those skilled in the art.

- Another aspect of the present invention is directed to methods of
- 5 identifying compounds which bind to either THP or nucleic acid molecules encoding THP, comprising contacting THP, or a nucleic acid molecule encoding the same, with a compound, and determining whether the compound binds THP, or a nucleic acid molecule encoding the same. Binding can be determined by binding assays which are well known to the skilled artisan, including, but not limited to, gel-shift assays, Western blots,
 - 10 radiolabeled competition assay, phage-based expression cloning, co-fractionation by chromatography, co-precipitation, cross linking, interaction trap/two-hybrid analysis, southwestern analysis, ELISA, and the like, which are described in, for example, *Current Protocols in Molecular Biology*, 1999, John Wiley & Sons, NY, which is incorporated herein by reference in its entirety. The compounds to be screened include (which may
 - 15 include compounds which are suspected to bind THP, or a nucleic acid molecule encoding the same), but are not limited to, extracellular, intracellular, biologic or chemical origin. The THP polypeptide or polynucleotide employed in such a test may either be free in solution, attached to a solid support, borne on a cell surface or located intracellularly. One skilled in the art can, for example, measure the formation of complexes between THP and
 - 20 the compound being tested. Alternatively, one skilled in the art can examine the diminution in complex formation between THP and its substrate caused by the compound being tested.

- Another aspect of the present invention is directed to methods of
- identifying compounds which modulate (*i.e.*, increase or decrease) activity of THP
 - 25 comprising contacting THP with a compound, and determining whether the compound modifies activity of THP. The activity in the presence of the test compound is measured and compared to the activity in the absence of the test compound. Where the activity of the sample containing the test compound is higher than the activity in the sample lacking the test compound, the compound will have increased activity. Similarly, where the
 - 30 activity of the sample containing the test compound is lower than the activity in the sample lacking the test compound, the compound will have inhibited activity.

- The present invention is particularly useful for screening compounds by using THP in any of a variety of drug screening techniques. The compounds to be screened include (which may include compounds which are suspected to modulate THP
- 35 activity), but are not limited to, extracellular, intracellular, biologic or chemical origin. The THP polypeptide employed in such a test may be in any form, preferably, free in solution, attached to a solid support, borne on a cell surface or located intracellularly. One

skilled in the art can, for example, measure the formation of complexes between THP and the compound being tested. Alternatively, one skilled in the art can examine the diminution in complex formation between THP and its substrate caused by the compound being tested.

- 5 The activity of THP polypeptide of the invention can be determined by, for example, measuring the ADP-ribosylating ability of the polypeptide in the presence and absence of the test compound (see, Simonin *et al.*, *J. Biol. Chem.*, 1993, 268, 8529, which is incorporated herein by reference in its entirety). In order to test for ADP-ribosylation activity, the polypeptide in question can be incubated with a radio-labeled substrate (³²P-NAD⁺) and a suitable protein target (such as TRF1). Co-migration of radio-labeled product with the protein target upon gel electrophoresis would indicate a positive result for ADP-ribosylation of the protein target and hence PARP activity in the polypeptide in question. Observation of larger and variably sized ADP-ribosylated products, when the assay is run in the presence of excess ³²P-NAD⁺, would also be expected for PARP catalytic activity. Negative controls are warranted to preclude the possibility of extraneous PARP activity from sources other than the polypeptide in question (*i.e.*, perform the assay with inactivated polypeptide or in the absence of polypeptide). Confirmation of the presence of ADP-ribose polymers on the protein target can be achieved by combination of the ADP-ribosylated product with a monoclonal antibody to poly(ADP-ribose) and subsequent detection of the antigen/antibody complex. In addition, PARP-like activity can be verified by addition of a specific PARP inhibitor (*i.e.*, 3-aminobenzamide) to assay runs and would result in significant inhibition of ADP-ribosylation activity.
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- In another embodiment of the invention, the activity of THP polypeptide of the invention can be determined by, for example, measuring ANK binding. The ANK domain in tankyrase binds to TRF1 and has significant structural homology with ankyrins, proteins which are generally involved in protein-protein interactions. Both the ANK domain of tankyrase and ankyrins contain 24 tandem arrays of the ANK motif of 33 amino acids and share subtle variations, including a shorter fifth ANK copy. So called "ankyrin-repeat proteins" have significantly fewer ANK copies, emphasizing the fact that tankyrase is a true ankyrin. Amino acid sequence analysis can be a primary means of identifying an ANK functional domain in any polypeptide and various binding and immunological assays (*i.e.*, immunoprecipitation) can potentially identify an ANK domain. In addition, TRF-1 binding assays can be performed with the polypeptides of the invention in the presence and absence of the test compound.
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- 35 Other assays can be used to examine enzymatic activity including, but not limited to, photometric, radiometric, HPLC, electrochemical, and the like, which are described in, for example, *Enzyme Assays: A Practical Approach*, eds. R. Eisinger and M.

J. Danson, 1992, Oxford University Press, which is incorporated herein by reference in its entirety. Representative assays which can be used for determining an activity of THP are described in, for example, Smith *et al.*, *Science*, 1998, 282, 1484-1487, Simonin *et al.*, *J. Biol. Chem.*, 1993, 268, 8529-8535, and U.S. Patent No. 5,874,444, each of which is
5 incorporated herein by reference in its entirety.

In preferred embodiments of the invention, methods of screening for compounds which modulate THP activity comprise contacting the compound with THP and assaying for the presence of a complex between the compound and THP. In such assays, THP is typically labeled. After suitable incubation, free THP is separated from
10 that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of the particular compound to bind to THP.

In another embodiment of the invention, high throughput screening for compounds having suitable binding affinity to THP is employed. Briefly, large numbers of different small peptide test compounds are synthesised on a solid substrate. The peptide
15 test compounds are contacted with THP and washed. Bound THP is then detected by methods well known in the art.

Purified polypeptides of the invention can also be coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies can be used to capture the protein and immobilize it on the solid
20 support.

Other embodiments of the invention comprise using competitive screening assays in which neutralizing antibodies capable of binding a polypeptide of the invention specifically compete with a test compound for binding to the polypeptide. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more
25 antigenic determinants with THP. Radiolabeled competitive binding studies are described in A.H. Lin *et al. Antimicrobial Agents and Chemotherapy*, 1997, vol. 41, no. 10. pp. 2127-2131, the disclosure of which is incorporated herein by reference in its entirety.

In other embodiments of the invention, the polypeptides of the invention are employed as a research tool for identification, characterization and purification of
30 interacting, regulatory proteins. Appropriate labels are incorporated into the polypeptides of the invention by various methods known in the art and the polypeptides are used to capture interacting molecules. For example, molecules are incubated with the labeled polypeptides, washed to removed unbound polypeptides, and the polypeptide complex is quantified. Data obtained using different concentrations of polypeptide are used to
35 calculate values for the number, affinity, and association of polypeptide with the protein complex.

Labeled polypeptides are also useful as reagents for the purification of molecules with which the polypeptide interacts including, but not limited to, inhibitors. In one embodiment of affinity purification, a polypeptide is covalently coupled to a chromatography column. Cells and their membranes are extracted, and various cellular
5 subcomponents are passed over the column. Molecules bind to the column by virtue of their affinity to the polypeptide. The polypeptide-complex is recovered from the column, dissociated and the recovered molecule is subjected to protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotides for cloning the corresponding gene from an appropriate cDNA library.

10 Alternatively, compounds may be identified which exhibit similar properties to THP of the invention, but which are smaller and exhibits a longer half time than THP in a human or animal body. When an organic compound is designed, a molecule according to the invention is used as a "lead" compound. The design of mimetics to known pharmaceutically active compounds is a well known approach in the
15 development of pharmaceuticals based on such "lead" compounds. Mimetic design, synthesis and testing are generally used to avoid randomly screening a large number of molecules for a target property. Furthermore, structural data deriving from the analysis of the deduced amino acid sequences encoded by the DNAs of the present invention are useful to design new drugs, more specific and therefore with a higher pharmacological
20 potency.

Comparison of the protein sequence of the present invention with the sequences present in all the available data bases showed a significant homology with the enzymatic PARP domain. Accordingly, computer modelling can be used to develop a putative tertiary structure of the proteins of the invention based on the available
25 information of other PARP domain proteins. Thus, novel enzyme inhibitors based on the predicted structure of THP can be designed.

In a particular embodiment, the novel molecules identified by the screening methods according to the invention are low molecular weight organic molecules, in which case a composition or pharmaceutical composition can be prepared thereof for oral intake,
30 such as in tablets. The compositions, or pharmaceutical compositions, comprising the nucleic acid molecules, vectors, polypeptides, antibodies and compounds identified by the screening methods described herein, can be prepared for any route of administration including, but not limited to, oral, intravenous, cutaneous, subcutaneous, nasal, intramuscular or intraperitoneal. The nature of the carrier or other ingredients will depend
35 on the specific route of administration and particular embodiment of the invention to be administered. Examples of techniques and protocols that are useful in this context are,

inter alia, found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A (ed.), 1980, which is incorporated herein by reference in its entirety.

5 The dosage of these low molecular weight compounds will depend on the disease state or condition to be treated and other clinical factors such as weight and condition of the human or animal and the route of administration of the compound. For treating human or animals, between approximately 0.5 mg/kg of body weight to 500 mg/kg of body weight of the compound can be administered. Therapy is typically administered at lower dosages and is continued until the desired therapeutic outcome is observed.

10 The present compounds and methods, including nucleic acid molecules, polypeptides, antibodies, compounds identified by the screening methods described herein, have a variety of pharmaceutical applications and may be used, for example, to treat or prevent unregulated cellular growth, such as cancer cell and tumour growth. In a particular embodiment, the present molecules are used in gene therapy. For a review of
15 gene therapy procedures, see e.g. Anderson, *Science*, 1992, 256, 808-813, which is incorporated herein by reference in its entirety.

The invention is further illustrated by way of the following examples which are intended to elucidate the invention. These examples are not intended, nor are they to be construed, as limiting the scope of the invention. It will be clear that the invention may
20 be practiced otherwise than as particularly described herein. Numerous modifications and variations of the present invention are possible in view of the teachings herein and, therefore, are within the scope of the invention.

Examples 1-3 presented below are actual whereas Examples 4-8 are prophetic.

25

EXAMPLES

Example 1: Identification of Homolog of Human Tankyrase

The nucleotide sequence of human tankyrase (GeneBank accession number AF082556), or the amino acid sequence deduced from it, was used as a query sequence
30 against the LifeSeqTM Assembled database (Incyte Pharmaceuticals). This database, which contains previously identified and annotated sequences, was searched for regions of similarity using Gapped BLAST, (Altschul *et al.*, *Nuc. Acids Res.*, 1997, 25, 3389, which is incorporated herein by reference in its entirety).

Briefly, the BLAST algorithm, which stands for Basic Local Alignment
35 Search Tool is suitable for determining sequence similarity (Altschul *et al.*, *J. Mol. Biol.*, 1990, 215, 403-410, which is incorporated herein by reference in its entirety). Software for performing BLAST analyses is publicly available through the National Center for

Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al.*, *supra*). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension for the word hits in each direction are halted when: 1) the cumulative alignment score falls off by the quantity X from its maximum achieved value; 2) the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or 3) the end of either sequence is reached. The Blast algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The Blast program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (*see* Henikoff *et al.*, *Proc. Natl. Acad. Sci. USA*, 1992, 89, 10915-10919, which is incorporated herein by reference in its entirety) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm (Karlin *et al.*, *Proc. Natl. Acad. Sci. USA*, 1993, 90, 5873-5877, which is incorporated herein by reference in its entirety) and Gapped BLAST perform a statistical analysis of the similarity between two sequences. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a Thp gene or cDNA if the smallest sum probability in comparison of the test nucleic acid to a Thp nucleic acid is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

Search of the LifeSeqTM Assembled database with the tankyrase-nucleotide sequence as a query sequence identified a template sequence, with ID # 232670.2, having a statistically significant overlapping homology to the query sequence. The sequence identified by template ID # 232670.2 had not previously been found to be homologous to human tankyrase.

The overlapping similarity extended from tankyrase AF082556 residues 2565 to 4165, that compared to template 232670 residues 1 to 1589, aligning with an overall DNA sequence similarity of 70%. The deduced amino acid sequence of AF082556, residues 2565 to 4165, and template 232670, residues 1 to 1589, aligned with a similarity of 80%. The similarity overlap included residues of tankyrase encoding the two most C-terminal ANK repeats, the SAM domain, and the PARP domain. In addition, template

232670.2 contained 2310 bp of sequence 3' to the overlap, not present in the published tankyrase sequence (AF082556).

- In the Incyte assembled database, the very 5' end sequence of template 232670.2 is given by Incyte clone 3206491 from Incyte library UTRSTMR01. Clone 5 3206491 was found to be 3697 bp long, and was sequenced fully by automated sequencing. The obtained sequence confirmed the sequence of template 232670.2 from nt. 1 to 3697.

Example 2: Cloning of Thp cDNA

- 10 To isolate a cDNA clone encoding full length THP, a DNA fragment corresponding to clone 3206491 nt 1 to 660, was used as a probe for hybridization screening of a phage cDNA library. The DNA fragment was amplified by the polymerase chain reaction (PCR) method. The PCR reaction mixture of 50 ml contained polymerase mixture (0.2 mM dNTPs, 1x PCR Buffer and 0.75 ml Expand High Fidelity Polymerase 15 (Roche Biochemicals)), 1 µg of 3206491 plasmid, and 50 pmoles of forward primer (GAGTATTTGTTACAACACGG) (SEQ ID NO:1) and 50 pmoles of reverse primer (AATCTCCTTCAGCTCCTT) (SEQ ID NO:2). Amplification was performed in an Applied Biosystems PE2400 thermocycler, using the following program: 95 C for 15 seconds, 52 C for 30 seconds and 72 C for 90 seconds; repeated for 25 cycles. The 20 amplified product was separated from the 3206491 plasmid by agarose gel electrophoresis, and purified by Qiaquick gel extraction kit (Qiagen).

- A lambda phage library containing cDNAs isolated from human brain (Stratagene cat. # 936213) cloned into lambda ZAPII phage-vector, was plated with *E. coli* XL-1 blue host, on 15 cm LB-agar plates at a density of 50,000 pfu per plate, and grown 25 overnight at 37 C; (plated as described by Sambrook et al., *supra*). Phage plaques were transferred to nylon membranes (Amersham Hybond NJ), denatured for 2 minutes in denaturation solution (0.5 M NaOH, 1.5 M NaCl), renatured for 5 minutes in renaturation solution (1 M Tris pH 7.5, 1.5 M NaCl), and washed briefly in 2xSSC (20x SSC: 3 M NaCl, 0.3 M Na-citrate). Filter membranes were dried and incubated at 80 C for 120 30 minutes to cross link the phage DNA to the membranes.

- The membranes were hybridized with a DNA probe prepared as described above. A DNA fragment (25 ng) was labeled with α -³²P-dCTP (NEN) using Rediprime random priming (Amersham Pharmacia Biotech), according to manufacturers instructions. Labeled DNA was separated from unincorporated nucleotides by S200 spin columns 35 (Amersham Pharmacia Biotech), denatured at 95 C for 5 minutes and kept on ice. The DNA-containing membranes (above) were pre-hybridised in 50 ml ExpressHyb (Clontech) solution at 68 C for 90 minutes. Subsequently, the labeled DNA probe was added to the

- hybridization solution, and the probe was left to hybridised to the membranes at 68 C for 70 minutes. The membranes were was five times in 2x SSC, 0.1% SDS at 42 C for 5 minutes each, and finally washed 30 minutes in 0.1x SSC, 0.2% SDS. Filters were exposed to Kodak XAR film (Eastman Kodak Company, Rochester, N.Y., USA) with an
- 5 intensifying screen at -80 C for 16 hours. One positive colony was isolated from the plates, and replated with about 1000 pfu on a 15 cm LB plate. Plating, plaque lift to filters and hybridization were performed as described above. Four positive phage plaques were isolated from this secondary screening.

- cDNA containing plasmids (pBluescript SK-) were rescued from the
- 10 isolated phages by *in vivo* excision by culturing XL-1 blue cells co-infected with the isolated phages and with the Excision helper phage, as described by manufacturer (Stratagene). XL-blue cells containing the plasmids were plated on LB plates and grown at 37 C for 16 hours. Colonies (18) from each plate were replated on LB plates and grown. One colony from each plate was stricken onto a nylon filter in an ordered array,
- 15 and the filter was placed on a LB plate to raise the colonies. The filter was then hybridized with a labeled probe as described above. Three positive colonies were selected and grown up in LB medium. Plasmid DNA was isolated from the three clones by Qiagen Midi Kit (Qiagen) according to the manufacturer's instructions. The size of the insert was determined by digesting the plasmid with the restriction enzymes NotI and SalI, which
- 20 established an insert size of approximately 4500 bp. The sequence of the entire insert was determined by automated sequencing on both strands of the plasmids, and is shown in SEQ ID NO:3. The sequence contains an open reading frame from positions 23 to 3520 (SEQ ID NO:4), encoding a polypeptide (1166 amino acid residues long), as shown in SEQ ID NO:5. Plasmid-containing SEQ ID NO:3 (pCF497) has been deposited with
- 25 American Type Culture Collection (ATCC) on May 10, 1999 and has been given Deposit No. PTA-35.

Example 3: Northern Blot Analysis

- Northern blots were performed to examine the expression of mRNA. The
- 30 sense orientation oligonucleotide 5'-CCCGAGAGCTGTTGAGGC (SEQ ID NO:6) with the antisense-orientation oligonucleotide 5'-CAATCTTTACTCTGTTATATCCT -3' (SEQ ID NO:7) were used as primers to amplify a portion of the THP cDNA sequence of SEQ ID NO:3. A fragment 606 basepairs from positions 96 to 701 long was amplified and used as a probe.

- 35 Multiple human tissue northern blot from Clontech (Human II # 7767-1) were hybridized with the probe. Pre-hybridization was carried out at 42 C for 4 hours in 5xSSC, 1x Denhardt's reagent, 0.1% SDS, 50% formamide, 250 mg/ml salmon sperm

DNA. Hybridization was performed overnight at 42 C in the same mixture with the addition of about 1.5×10^6 cpm/ml of labeled probe.

The probe was labeled with α - ^{32}P -dCTP by Rediprime DNA labelling system (Amersham Pharmacia), purified on Nick Column (Amersham Pharmacia) and added to the hybridization solution. The filters were washed several times at 42 C in 0.2x SSC, 0.1% SDS. Filters were exposed to Kodak XAR film (Eastman Kodak Company, Rochester, N.Y., USA) with intensifying screen at -80 C.

A single 7 kb mRNA is expressed in all tissues analysed, including heart, brain placenta, lung, liver, skeletal muscle, kidney and pancreas. The expression is rather constant in all tissues, except for placenta and skeletal muscle, which show an approximately 2-fold increase in expression levels over the other tissues. Equal loading of all the lanes was verified by filter hybridisation with a human GAPDH probe (data not shown).

15

Example 4: Expression of THP in Mammalian Cells

1. Expression of THP in 293 cells

For expression of THP in mammalian cells 293 (transformed human, primary embryonic kidney cells), a plasmid bearing the relevant THP coding sequence is prepared, using vector pcDNA3.1 myc-his (Invitrogen). The plasmid contains nucleotides 17 through 3513 of SEQ ID NO:3. Vector pcDNA3.1 contains the *c-myc* epitope for detection of the recombinant protein with the anti-myc antibody, a C-terminal polyhistidine for purification with nickel chelate chromatography, and a Neomycin resistant gene for selection of stable transfectants. The forward primer for amplification of this THP cDNA is: 5'-

AAGCGGCCGCGATTATGGAAAGGATCATGTCTGGGTCGCCGCT-3' (SEQ ID NO:8) which contains a 5' extension of 19 nucleotides to introduce the NotI cloning site and 22 nucleotides matching the THP sequence (nucleotides 17 through 38 of SEQ ID NO:3). The reverse primer is: 5'-AAGGATCCACCATACCTTCAGGCCT-3' (SEQ ID NO:9) which contains a 5' extension of 8 nucleotides to introduce an BamHI restriction site for cloning and 17 nucleotides corresponding to the reverse complement of the THP sequence from bases 3497 to 3513 of SEQ ID NO:3. The PCR conditions are 55 C as the annealing temperature. The PCR product is gel purified and cloned into the NotI-BamHI sites of the vector.

35

The DNA is purified using Qiagen chromatography columns and transfected into 293 cells using SUPERFACT transfection media (Qiagen). Transiently transfected cells are tested for expression after 24 hours of transfection, using western

blots probed with antiHis and anti-THP peptide antibodies. Permanently transfected cells are selected with G418 and propagated. Production of the recombinant protein is detected from cells by Western blots probed with anti-His, anti-Myc or anti-THP peptide antibodies.

5

2. Expression of THP in COS cells

For expression of the THP in COS7 cells, a polynucleotide molecule having the sequence given as nucleotides 17 through 3513 of SEQ ID NO:3 was cloned into vector pSecTag2A. Vector pSecTag2A contains the murine IgK chain leader sequence for secretion, the *c-myc* epitope for detection of the recombinant protein with the anti-myc antibody, a C-terminal polyhistidine for purification with nickel chelate chromatography, and a Zeocin resistant gene for selection of stable transfectants.

10

The forward primer is 5'-

AAAAGCTTTATGGAAAGGATCATGTCGGGT

CGCCGCTGC-3' (SEQ ID NO:10). The first 15 nucleotides of this primer constitute a 5' extension which introduces an HindIII restriction site for cloning, followed by 22 nucleotides which correspond to nucleotide residues 17-38 of the sequence given in SEQ ID NO: 3. The reverse primer is SEQ ID NO:9. This primer contains a 5'- extension of 8 nucleotides which introduces a BamHI cloning site followed by 17 nucleotides which correspond to the reverse complement of bases 3497 to 3513 of the sequence given in SEQ ID NO:3.

20

The PCR consists of an initial denaturation step of 5 min at 95°C, 30 cycles of 30 sec denaturation at 95°C, 30 sec annealing at 58°C and 30 sec extension at 72°C, followed by 5 min extension at 72°C. The PCR product is gel purified and ligated into the XbaI and SalI sites of vector p3-CI. This construct is transformed into *E. coli* cells for amplification and DNA purification. The DNA is purified with Qiagen chromatography columns and transfected into COS 7 cells using Lipofectamine reagent from BRL, following the manufacturer's protocols. Forty eight and 72 hours after transfection, the media and the cells are tested for recombinant protein expression.

30

THP expressed from a COS cell culture can be purified by concentrating the cell-growth media to about 10 mg of protein/ml, and purifying the protein by, for example, chromatography. Purified THP is concentrated to 0.5 mg/ml in an Amicon concentrator fitted with a YM-10 membrane and stored at -80°C.

35 Example 5: Expression of THP in Insect Cells

For expression of THP in a baculovirus system, a polynucleotide molecule having the sequence given as nucleotides 17 through 3513 of SEQ ID NO:3 was amplified

by PCR. The forward primer is SEQ ID NO:8. The first 19 nucleotides of this primer constitute a 5' extension which adds the NotI cloning site, followed by followed by 22 nucleotides which correspond to nucleotide residues 17 through 38 of the sequence given in SEQ ID NO:3. The reverse primer is SEQ ID NO:9. The first 8 nucleotides of this
5 primer constitute a 5' extension which introduces the BamHI cloning site, followed by followed by 17 nucleotides which correspond to the reverse complement of nucleotide residues 3497 through 3513 of the sequence given in SEQ ID NO:3.

The PCR product is gel purified, digested with NdeI and KpnI, and cloned into the corresponding sites of vector pAcHTL-A (Pharmingen, San Diego, CA). The
10 pAcHTL expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV), and a 6XHis tag upstream from the multiple cloning site. A protein kinase site for phosphorylation and a thrombin site for excision of the recombinant protein precede the multiple cloning site is also present. Of course, many other baculovirus vectors could be used in place of pAcHTL-A, such as
15 pAc373, pVL941 and pAcIM1. Other suitable vectors for the expression of THP polypeptides can be used, provided that the vector construct includes appropriately located signals for transcription, translation, and trafficking, such as an in-frame AUG and a signal peptide, as required. Such vectors are described in Luckow *et al.*, Virology 170:31-39, among others.

20 The virus is grown and isolated using standard baculovirus expression methods, such as those described in Summers *et al.* (*A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station Bulletin No. 1555 (1987)).

In a preferred embodiment, pAcHLT-A containing the THP gene is
25 introduced into baculovirus using the "BaculoGold" transfection kit (Pharmingen, San Diego, CA) using methods established by the manufacturer. Individual virus isolates are analyzed for protein production by radiolabeling infected cells with ³⁵S-methionine at 24 hours post infection. Infected cells are harvested at 48 hours post infection, and the labeled proteins are visualized by SDS-PAGE. Viruses exhibiting high expression levels
30 can be isolated and used for scaled up expression.

For expression of the THP polypeptide in a Sf9 cells, a polynucleotide molecule having the sequence given as nucleotides 17 through 3513 of SEQ ID NO:3 is amplified by PCR using the primers and methods described above for baculovirus expression. The THP cDNA is cloned into vector pAcHLT-A (Pharmingen) for
35 expression in Sf9 insect. The insert is cloned into the NotI and BamHI sites, after elimination of an internal NdeI site (using the same primers described above for expression in baculovirus). DNA is purified with Qiagen chromatography columns and

expressed in Sf9 cells. Preliminary Western blot experiments from non purified plaques are tested for the presence of the recombinant protein of the expected size which reacted with the THP-specific antibody. These results are confirmed after further purification and expression optimization in HiG5 cells.

5

Example 6: Interaction Trap/Two-Hybrid System

- In order to assay for THP-interacting proteins, the interaction trap/two-hybrid library screening method can be used. This assay was first described in Fields, *et al.*, *Nature*, 1989, 340, 245, which is incorporated herein by reference in its entirety. A
- 10 protocol is published in *Current Protocols in Molecular Biology* 1999, John Wiley & Sons, NY and Ausubel, F. M. *et al.* 1992, *Short protocols in molecular biology*, fourth edition, Greene and Wiley-interscience, NY, which is incorporated herein by reference in its entirety. Kits are available from Clontech, Palo Alto, CA (Matchmaker Two-Hybrid System 3).
- 15 A fusion of the nucleotide sequences encoding all or partial THP and the yeast transcription factor GAL4 DNA-binding domain (DNA-BD) is constructed in an appropriate plasmid (ie. pGBKT7) using standard subcloning techniques. Similarly, a GAL4 active domain (AD) fusion library is constructed in a second plasmid (ie. pGADT7) from cDNA of potential THP-binding proteins (for protocols on forming cDNA libraries,
- 20 see Sambrook *et al.* 1989, *Molecular cloning: a laboratory manual*, second edition, Cold Spring Harbor Press, Cold Spring Harbor, NY), which is incorporated herein by reference in its entirety. The DNA-BD/THP fusion construct is verified by sequencing, and tested for autonomous reporter gene activation and cell toxicity, both of which would prevent a successful two-hybrid analysis. Similar controls are performed with the AD/library fusion
- 25 construct to ensure expression in host cells and lack of transcriptional activity. Yeast cells are transformed (*ca.* 10^5 transformants/mg DNA) with both the THP and library fusion plasmids according to standard procedure (Ausubel, *et al.*, 1992, *Short protocols in molecular biology*, fourth edition, Greene and Wiley-interscience, NY, which is incorporated herein by reference in its entirety). In vivo binding of DNA-BD/THP with
- 30 AD/library proteins results in transcription of specific yeast plasmid reporter genes (ie. lacZ, HIS3, ADE2, LEU2). Yeast cells are plated on nutrient-deficient media to screen for expression of reporter genes. Colonies are dually assayed for β -galactosidase activity upon growth in Xgal (5-bromo-4-chloro-3-indolyl- β -D-galactoside) supplemented media (filter assay for β -galactosidase activity is described in Breeden, *et al.*, *Cold Spring Harb. Symp.*
- 35 *Quant. Biol.*, 1985, 50, 643, which is incorporated herein by reference in its entirety). Positive AD-library plasmids are rescued from transformants and reintroduced into the original yeast strain as well as other strains containing unrelated DNA-BD fusion proteins.

to confirm specific THP/library protein interactions. Insert DNA is sequenced to verify the presence of an open reading frame fused to GAL4 AD and to determine the identity of the THP-binding protein.

5 **Example 7: Mobility Shift DNA-Binding Assay Using Gel Electrophoresis**

A gel electrophoresis mobility shift assay can rapidly detect specific protein-DNA interactions. Protocols are widely available in such manuals as Sambrook et al. 1989, *Molecular cloning: a laboratory manual*, second edition, Cold Spring Harbor Press, Cold Spring Harbor, NY and Ausubel, F. M. et al. 1992, *Short protocols in molecular biology*, fourth edition, Greene and Wiley-interscience, NY, each of which is incorporated herein by reference in its entirety.

Probe DNA(<300 bp) is obtained from synthetic oligonucleotides, restriction endonuclease fragments, or PCR fragments and end-labeled with ^{32}P . An aliquot of purified THP (ca. 15 μg) or crude THP extract (ca. 15 ng) is incubated at
15 constant temperature (in the range 22-37 C) for at least 30 minutes in 10-15 μl of buffer (i.e., TAE or TBE, pH 8.0-8.5) containing radiolabeled probe DNA, nonspecific carrier DNA (ca. 1 μg), BSA (300 $\mu\text{g/ml}$), and 10% (v/v) glycerol. The reaction mixture is then loaded onto a polyacrylamide gel and run at 30-35 mA until good separation of free probe DNA from protein-DNA complexes occurs. The gel is then dried and bands
20 corresponding to free DNA and protein-DNA complexes are detected by autoradiography.

Example 8: Assay to Identify Compounds that Modulate THP Activity

THP is capable of auto-poly[ADP]-ribosylation through the activity of the PARP domain. To screen for this THP activity, THP expressed in baculovirus is used (see,
25 Example 5). Four μg of THP is incubated in 100 μl of assay buffer (50 mM Tris, pH 8.0, 4 mM MgCl_2 , 0.2 mM DTT) together with 1.3 μM ^{32}P -labeled NAD^+ , at 25 C for 30 minutes. Incorporation of ^{32}P -labeled NAD^+ is measured by TCA precipitation, by addition of 20% TCA, and collection by filtration of insoluble material onto glass filters. The filters are counted by scintillation counting in a fluid beta-counter, and the resulting
30 counts are used as a measure for THP PARP activity. This same assay can also be used, for example, to screen large libraries of chemical compounds for small molecules that are THP PARP inhibitors.

Some of the preferred embodiments of the invention described above are outlined below and include, but are not limited to, the following embodiments. As those
35 skilled in the art will appreciate, numerous changes and modifications may be made to the preferred embodiments of the invention without departing from the spirit of the invention.

It is intended that all such variations fall within the scope of the invention. The entire disclosure of each publication cited herein is hereby incorporated by reference.

[illegible]

WHAT IS CLAIMED IS:

- 1 An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
 - 5 a) SEQ ID NO:3, or a fragment thereof;
 - b) SEQ ID NO:4, or a fragment thereof;
 - c) a sequence homologous to SEQ ID NO:3 or SEQ ID NO:4, or a fragment thereof;
 - d) a sequence that encodes a polypeptide comprising SEQ ID NO:5, or a fragment thereof; and
 - 10 e) a sequence that encodes a polypeptide comprising an amino acid sequence homologous to SEQ ID NO:5, or a fragment thereof;said nucleic acid molecule encoding at least a portion of a tankyrase homolog protein.
- 15 2. The nucleic acid molecule of claim 1 wherein said nucleic acid molecule is DNA.
3. The nucleic acid molecule of claim 1 wherein said nucleic acid molecule is RNA.
4. The nucleic acid molecule of claim 2 wherein said nucleotide sequence comprises
20 SEQ ID NO:4.
5. An isolated nucleic acid molecule comprising a nucleotide sequence complementary to at least a portion of SEQ ID NO:3 or SEQ ID NO:4, a complement of said nucleic acid molecule encoding at least a portion of a tankyrase homolog protein.
25
6. The nucleic acid molecule of claim 5 wherein said molecule is an antisense oligonucleotide directed to SEQ ID NO:3 or SEQ ID NO:4.
7. The nucleic acid molecule of claim 6 wherein said oligonucleotide is directed to a
30 regulatory region of SEQ ID NO:3 or SEQ ID NO:4.
8. An expression vector comprising a nucleic acid molecule of claim 1 or 5.
9. An expression vector of claim 8, wherein said nucleic acid molecule comprises
35 SEQ ID NO:4.
10. The vector of claim 8 wherein said vector is a plasmid.
11. The vector of claim 8 wherein said vector is a viral particle.
- 40 12. The vector of claim 11 wherein said vector is selected from the group consisting of adenoviruses, parvoviruses, herpesviruses, poxviruses, adeno-associated viruses, Semliki Forest viruses, vaccinia viruses, and retroviruses.
- 45 13. The vector of claim 8 wherein said nucleic acid molecule is operably connected to a promoter selected from the group consisting of simian virus 40, mouse mammary tumor virus, long terminal repeat of human immunodeficiency virus, maloney virus, cytomegalovirus immediate early promoter, Epstein Barr virus, rous sarcoma virus, human

actin, human myosin, human hemoglobin, human muscle creatine, and human metallothionein.

14. A host cell transformed with a vector of claim 8.
15. The transformed host cell of claim 14 wherein said cell is a bacterial cell.
16. ~~The transformed host cell of claim 15 wherein said bacterial cell is *E. coli*.~~
17. The transformed host cell of claim 14 wherein said cell is a yeast.
18. The transformed host cell of claim 17 wherein said yeast is *S. cerevisiae*.
19. The transformed host cell of claim 14 wherein said cell is an insect cell.
20. The transformed host cell of claim 19 wherein said insect cell is *S. frugiperda*.
21. The transformed host cell of claim 14 wherein said cell is a mammalian cell.
22. The transformed host cell of claim 21 wherein mammalian cell is selected from the group consisting of chinese hamster ovary cells, HeLa cells, African green monkey kidney cells, human 293 cells, and murine 3T3 fibroblasts.
23. A method of producing a polypeptide comprising SEQ ID NO:5, or a homolog or fragment thereof, comprising the steps of:
 - a) introducing a recombinant expression vector of claim 7 into a compatible host cell;
 - b) growing said host cell under conditions for expression of said polypeptide; and
 - c) recovering said polypeptide.
24. The method of claim 23 wherein said host cell is lysed and said polypeptide is recovered from the lysate of said host cell.
25. The method of claim 23 wherein said polypeptide is recovered by purifying the culture medium without lysing said host cell.
26. A composition comprising a nucleic acid molecule of claim 1 or 5 and an acceptable carrier or diluent.
27. A composition comprising a recombinant expression vector of claim 8 and an acceptable carrier or diluent.
28. An isolated polypeptide encoded by a nucleic acid molecule of claim 1.
29. The polypeptide of claim 28 wherein said polypeptide comprises SEQ ID NO:5.
30. The polypeptide of claim 28 wherein said polypeptide comprises an amino acid sequence homologous to SEQ ID NO:5.

31. The polypeptide of claim 30 wherein said sequence homologous to SEQ ID NO:5 comprises at least one conservative amino acid substitution compared to SEQ ID NO:5.
32. The polypeptide of claim 28 wherein said polypeptide comprises a fragment of, SEQ ID NO:5.
33. A composition comprising a polypeptide of claim 28 and an acceptable carrier or diluent.
34. An isolated antibody which binds to an epitope on a polypeptide of claim 28.
35. The antibody of claim 34 wherein said antibody is a monoclonal antibody.
36. A composition comprising an antibody of claim 34 and an acceptable carrier or diluent.
37. A kit comprising an antibody which binds to a polypeptide of claim 28 and a negative control antibody.
38. The kit of claim 37 further comprising an additional kit component.
39. The kit of claim 38 wherein said additional kit component comprises instructions.
40. A kit comprising a nucleic acid molecule of claim 1 or 5.
41. The kit of claim 40 further comprising an additional kit component.
42. The kit of claim 41 wherein said additional kit component comprises instructions.
43. A kit comprising a polypeptide of claim 28.
44. The kit of claim 43 further comprising an additional kit component.
45. The kit of claim 44 wherein said additional kit component comprises instructions.
46. A method of inducing an immune response in a mammal against a polypeptide of claim 28 comprising administering to said mammal an amount of said polypeptide sufficient to induce said immune response.
47. A method for identifying a compound which binds tankyrase homolog protein (THP) comprising the steps of:
a) contacting THP with a compound; and
b) determining whether said compound binds THP.
48. The method of claim 47 wherein binding of said compound to THP is determined by a protein binding assay.

49. The method of claim 48 wherein said protein binding assay is selected from the group consisting of a gel-shift assay, Western blot, radiolabeled competition assay, phage-based expression cloning, co-fractionation by chromatography, co-precipitation, cross linking, interaction trap/two-hybrid analysis, southwestern analysis, and ELISA.

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50. A method for identifying a compound which binds a nucleic acid molecule encoding tankyrase homolog protein (THP) comprising the steps of:

a) contacting said nucleic acid molecule encoding THP with a compound; and

10

b) determining whether said compound binds said nucleic acid molecule.

51. The method of claim 50 wherein binding is determined by a gel-shift assay.

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52. A method for identifying a compound which modulates the activity of tankyrase homolog protein (THP) comprising the steps of:

a) contacting THP with a compound; and

b) determining whether THP activity has been modulated.

20

53. The method of claim 52 wherein said activity is ADP-ribosylation.

54.

The method of claim 52 wherein said activity is TRF1 binding.

55.

A compound identified by the method of claim 47.

25

56.

A compound identified by the method of claim 50.

57.

A compound identified by the method of claim 52.

[illegible]

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Jens Berthelsen, Salvatore Toma, Antonella
Isacchi

Group Art Unit: Not Yet
Assigned

For: TANKYRASE HOMOLOG PROTEIN
(THP), NUCLEIC ACIDS, AND
METHODS RELATED TO THE SAME

Examiner: Not Yet Assigned

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a

☒ Utility Patent ☐ Design Patent

is sought on the invention, whose title appears above, the specification of which:

- ☒ is attached hereto.
☐ was filed on _____ as Serial No. _____
☐ said application having been amended on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to be material to the patentability of this application in accordance with 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a-d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below

any foreign application for patent or inventor's certificate having a filing date before that of any application on which priority is claimed:

Priority Claimed (If X'd)	Country	Serial Number	Date Filed
<input type="checkbox"/>	_____	_____	_____
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<input type="checkbox"/>	_____	_____	_____

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to be material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Serial Number	Date Filed	Patented/Pending/Abandoned
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Serial Number	Date Filed
_____	_____

I hereby appoint the following persons of the firm of **WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS LLP**, One Liberty Place - 46th Floor, Philadelphia, Pennsylvania 19103 as attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Suzanne E. Miller

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Mark J. Rosen

Reg. No. 39,822

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**WOODCOCK WASHBURN KURTZ
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Facsimile No.: (215) 568-3439

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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	Citizenship: _____

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ttc gta agg nat ctt gga ctt gag cac cta atg gat ata ttt gag aga 2688
 Phe Val Arg Xaa Leu Gly Leu Glu His Leu Met Asp Ile Phe Glu Arg
 885 890 895

gaa cag atc act ttg gat gta tta gtt gag atg ggg cac aag gag ctg 2736
 Glu Gln Ile Thr Leu Asp Val Leu Val Glu Met Gly His Lys Glu Leu
 900 905 910

aag gag att gga atc aat gct tat gga cat agg cac aaa cta att aaa 2784
 Lys Glu Ile Gly Ile Asn Ala Tyr Gly His Arg His Lys Leu Ile Lys
 915 920 925

gga gtc gag aga ctt atc tcc gga caa caa ggt ctt aac cca tat tta 2832
 Gly Val Glu Arg Leu Ile Ser Gly Gln Gln Gly Leu Asn Pro Tyr Leu
 930 935 940

act ttg aac acc tct ggt agt gga aca att ctt ata gat ctg tct cct 2880
 Thr Leu Asn Thr Ser Gly Ser Gly Thr Ile Leu Ile Asp Leu Ser Pro
 945 950 955 960

gat gat aaa gag ttt cag tct gtg gag gaa gag atg caa agt aca gtt 2928
 Asp Asp Lys Glu Phe Gln Ser Val Glu Glu Glu Met Gln Ser Thr Val
 965 970 975

cga gag cac aga gat gga ggt cat gca ggt gga atc ttc aac aga tac 2976
 Arg Glu His Arg Asp Gly Gly His Ala Gly Gly Ile Phe Asn Arg Tyr
 980 985 990

aat att ctc aag att cag aag gtt tgt aac aag aaa cta tgg gaa aga 3024
 Asn Ile Leu Lys Ile Gln Lys Val Cys Asn Lys Lys Leu Trp Glu Arg

995 1000 1005

tac act cac cgg aga aaa gaa gtt tct gaa gaa aac cac aac cat gcc 3072
Tyr Thr His Arg Arg Lys Glu Val Ser Glu Glu Asn His Asn His Ala
1010 1015 1020

aat gaa cga atg cta ttt cat ggg tct cct ttt gtg aat gca att atc 3120
Asn Glu Arg Met Leu Phe His Gly Ser Pro Phe Val Asn Ala Ile Ile
1025 1030 1035 1040

cac aaa ggc ttt gat gaa agg cat gcg tac ata ggt ggt atg ttt gga 3168
His Lys Gly Phe Asp Glu Arg His Ala Tyr Ile Gly Gly Met Phe Gly
1045 1050 1055

gct ggc att tat ttt gct gaa aac tct tcc aaa agc aat caa tat gta 3216
Ala Gly Ile Tyr Phe Ala Glu Asn Ser Ser Lys Ser Asn Gln Tyr Val
1060 1065 1070

tat gga att gga gga ggt act ggg tgt cca gtt cac aaa gac aga tct 3264
Tyr Gly Ile Gly Gly Gly Thr Gly Cys Pro Val His Lys Asp Arg Ser
1075 1080 1085

tgt tac att tgc cac agg cag ctg ctc ttt tgc cgg gta acc ttg gga 3312
Cys Tyr Ile Cys His Arg Gln Leu Leu Phe Cys Arg Val Thr Leu Gly
1090 1095 1100

aag tct ttc ctg cag ttc agt gca atg aaa atg gca cat tct cct cca 3360
Lys Ser Phe Leu Gln Phe Ser Ala Met Lys Met Ala His Ser Pro Pro
1105 1110 1115 1120

ggt cat cac tca gtc act ggt agg ccc agt gta aat ggc cta gca tta 3408
Gly His His Ser Val Thr Gly Arg Pro Ser Val Asn Gly Leu Ala Leu
1125 1130 1135

gct gaa tat gtt att tac aga gga gaa cag gct tat cct gag tat tta 3456
Ala Glu Tyr Val Ile Tyr Arg Gly Glu Gln Ala Tyr Pro Glu Tyr Leu
1140 1145 1150

att act tac cag att atg agg cct gaa ggt atg gtc gat gga 3498
Ile Thr Tyr Gln Ile Met Arg Pro Glu Gly Met Val Asp Gly
1155 1160 1165

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<212> PRT

<213> Homo sapiens

<400> 5

Met Ser Gly Arg Arg Cys Ala Gly Gly Gly Ala Ala Cys Ala Ser Ala
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Ala Ala Glu Ala Val Glu Pro Ala Ala Arg Glu Leu Phe Glu Ala Cys
20 25 30

Arg Asn Gly Asp Val Glu Arg Val Lys Arg Leu Val Thr Pro Glu Lys
35 40 45

Val Asn Ser Arg Asp Thr Ala Gly Arg Lys Ser Thr Pro Leu His Leu
50 55 60

Ala Ala Gly Phe Gly Arg Lys Asp Val Val Glu Tyr Leu Leu Gln Asn
65 70 75 80

Gly Ala Asn Val Gln Ala Arg Asp Asp Gly Gly Leu Ile Pro Leu His
85 90 95

Asn Ala Cys Ser Phe Gly His Ala Glu Val Val Asn Leu Leu Leu Arg
100 105 110

His Gly Ala Asp Pro Asn Ala Arg Asp Asn Trp Asn Tyr Thr Pro Leu
115 120 125

His Glu Ala Ala Ile Lys Gly Lys Ile Asp Val Cys Ile Val Leu Leu
130 135 140

Gln His Gly Ala Glu Pro Thr Ile Arg Asn Thr Asp Gly Arg Thr Ala
145 150 155 160

Leu Asp Leu Ala Asp Pro Ser Ala Lys Ala Val Leu Thr Gly Glu Tyr
165 170 175

Lys Lys Asp Glu Leu Leu Glu Ser Ala Arg Ser Gly Asn Glu Glu Lys
180 185 190

Met Met Ala Leu Leu Thr Pro Leu Asn Val Asn Cys His Ala Ser Asp
195 200 205

Gly Arg Lys Ser Thr Pro Leu His Leu Ala Ala Gly Tyr Asn Arg Val
210 215 220

Lys Ile Val Gln Leu Leu Leu Gln His Gly Ala Asp Val His Ala Lys
225 230 235 240

Asp Lys Gly Asp Leu Val Pro Leu His Asn Ala Cys Ser Tyr Gly His
245 250 255

Tyr Glu Val Thr Glu Leu Leu Val Lys His Gly Ala Cys Val Asn Ala
260 265 270

Met Asp Leu Trp Gln Phe Thr Pro Leu His Glu Ala Ala Ser Lys Asn
275 280 285

Arg Val Glu Val Cys Ser Leu Leu Leu Ser Tyr Gly Ala Asp Pro Thr
290 295 300

Leu Leu Asn Cys His Asn Lys Ser Ala Ile Asp Leu Ala Pro Thr Pro
305 310 315 320

Gln Leu Lys Glu Arg Leu Ala Tyr Glu Phe Lys Gly His Ser Leu Leu
325 330 335

Gln Ala Ala Arg Glu Ala Asp Val Thr Arg Ile Lys Lys His Leu Ser
340 345 350

Leu Glu Met Val Asn Phe Lys His Pro Gln Thr His Glu Thr Ala Xaa
355 360 365

His Cys Ala Ala Ala Ser Pro Tyr Pro Lys Arg Lys Gln Ile Cys Glu
370 375 380

Leu Leu Leu Arg Lys Gly Ala Xaa Ile Asn Glu Lys Thr Lys Glu Phe
385 390 395 400

Leu Thr Pro Leu His Val Ala Ser Glu Lys Ala His Asn Asp Xaa Val
405 410 415

Glu Val Val Val Lys His Glu Ala Lys Val Asn Ala Leu Asp Asn Leu
420 425 430

Gly Gln Thr Ser Leu His Arg Ala Ala Tyr Cys Gly His Leu Gln Thr
435 440 445

Cys Arg Leu Leu Leu Ser Tyr Gly Cys Asp Pro Asn Ile Ile Ser Leu
450 455 460

Gln Gly Phe Thr Ala Leu Gln Met Gly Asn Glu Asn Val Gln Gln Leu
465 470 475 480

Leu Gln Glu Gly Ile Ser Leu Gly Asn Ser Glu Ala Asp Arg Gln Leu

485

490

495

Leu Glu Ala Ala Lys Ala Gly Asp Val Glu Thr Val Lys Lys Leu Cys
500 505 510

Thr Val Gln Ser Val Asn Cys Arg Asp Ile Glu Gly Arg Gln Ser Thr
515 520 525

Pro Leu His Phe Ala Ala Gly Tyr Asn Arg Val Ser Val Val Glu Tyr
530 535 540

Leu Leu Gln His Gly Ala Asp Val His Ala Lys Asp Lys Xaa Xaa Leu
545 550 555 560

Val Pro Leu His Asn Ala Cys Ser Tyr Gly His Tyr Glu Val Ala Glu
565 570 575

Leu Leu Val Lys His Gly Ala Val Val Asn Val Ala Asp Leu Trp Lys
580 585 590

Phe Thr Pro Leu His Glu Ala Ala Ala Lys Gly Lys Tyr Glu Ile Cys
595 600 605

Lys Leu Leu Leu Gln His Gly Ala Asp Pro Thr Lys Lys Asn Arg Asp
610 615 620

Gly Asn Thr Pro Leu Asp Leu Val Lys Asp Gly Asp Thr Asp Ile Gln
625 630 635 640

Asp Leu Leu Arg Gly Asp Ala Ala Leu Leu Asp Ala Ala Lys Lys Ser
645 650 655

Cys Leu Ala Arg Val Lys Lys Leu Ser Ser Pro Asp Asn Val Asn Cys
660 665 670

Arg Asp Thr Gln Gly Arg His Ser Thr Pro Leu His Leu Ala Ala Gly
675 680 685

Tyr Asn Asn Leu Glu Val Ala Glu Tyr Leu Leu Gln His Gly Ala Asp
690 695 700

Val Asn Ala Gln Asp Lys Gly Gly Leu Ile Pro Leu His Asn Ala Ala
705 710 715 720

Ser Tyr Gly His Val Asp Val Ala Ala Leu Leu Ile Lys Tyr Asn Ala
725 730 735

Cys Val Asn Ala Thr Asp Lys Trp Ala Phe Thr Pro Leu His Glu Ala
740 745 750

Ala Gln Lys Gly Arg Thr Gln Leu Cys Ala Leu Xaa Leu Ala His Gly
755 760 765

Ala Asp Pro Thr Leu Lys Asn Gln Glu Gly Gln Thr Pro Leu Asp Leu
770 775 780

Val Ser Ala Asp Asp Val Ser Ala Leu Leu Thr Ala Ala Met Pro Pro
785 790 795 800

Ser Ala Leu Pro Ser Cys Tyr Lys Pro Gln Val Leu Asn Gly Val Arg
805 810 815

Ser Pro Gly Ala Thr Ala Asp Ala Leu Ser Ser Gly Pro Ser Ser Pro
820 825 830

Ser Ser Leu Ser Ala Ala Ser Ser Leu Asp Asn Leu Ser Gly Ser Phe
835 840 845

Ser Glu Leu Ser Ser Val Val Ser Ser Ser Gly Thr Glu Gly Ala Ser
850 855 860

Ser Leu Glu Lys Lys Glu Val Pro Gly Val Asp Phe Ser Ile Thr Gln
865 870 875 880

Phe Val Arg Xaa Leu Gly Leu Glu His Leu Met Asp Ile Phe Glu Arg
885 890 895

Glu Gln Ile Thr Leu Asp Val Leu Val Glu Met Gly His Lys Glu Leu
900 905 910

Lys Glu Ile Gly Ile Asn Ala Tyr Gly His Arg His Lys Leu Ile Lys
915 920 925

Gly Val Glu Arg Leu Ile Ser Gly Gln Gln Gly Leu Asn Pro Tyr Leu
930 935 940

Thr Leu Asn Thr Ser Gly Ser Gly Thr Ile Leu Ile Asp Leu Ser Pro
945 950 955 960

Asp Asp Lys Glu Phe Gln Ser Val Glu Glu Glu Met Gln Ser Thr Val
965 970 975

Arg Glu His Arg Asp Gly Gly His Ala Gly Gly Ile Phe Asn Arg Tyr

980

985

990

Asn Ile Leu Lys Ile Gln Lys Val Cys Asn Lys Lys Leu Trp Glu Arg
 995 1000 1005

Tyr Thr His Arg Arg Lys Glu Val Ser Glu Glu Asn His Asn His Ala
 1010 1015 1020

Asn Glu Arg Met Leu Phe His Gly Ser Pro Phe Val Asn Ala Ile Ile
 1025 1030 1035 1040

His Lys Gly Phe Asp Glu Arg His Ala Tyr Ile Gly Gly Met Phe Gly
 1045 1050 1055

Ala Gly Ile Tyr Phe Ala Glu Asn Ser Ser Lys Ser Asn Gln Tyr Val
 1060 1065 1070

Tyr Gly Ile Gly Gly Gly Thr Gly Cys Pro Val His Lys Asp Arg Ser
 1075 1080 1085

Cys Tyr Ile Cys His Arg Gln Leu Leu Phe Cys Arg Val Thr Leu Gly
 1090 1095 1100

Lys Ser Phe Leu Gln Phe Ser Ala Met Lys Met Ala His Ser Pro Pro
 1105 1110 1115 1120

Gly His His Ser Val Thr Gly Arg Pro Ser Val Asn Gly Leu Ala Leu
 1125 1130 1135

Ala Glu Tyr Val Ile Tyr Arg Gly Glu Gln Ala Tyr Pro Glu Tyr Leu
 1140 1145 1150

Ile Thr Tyr Gln Ile Met Arg Pro Glu Gly Met Val Asp Gly
 1155 1160 1165

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<213> Artificial Sequence

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<223> Description of Artificial Sequence: PCR Primers

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19

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<212> DNA
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<400> 7
caatctttac tctgttatat cct 23

<210> 8
<211> 41
<212> DNA
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<400> 8
aagcggccgc attatggaaa ggatcatgtc gggtcgccgc t 41

<210> 9
<211> 25
<212> DNA
<213> Artificial Sequence

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<400> 9
aaggatccac cataccttca ggcct 25

<210> 10
<211> 39
<212> DNA
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<220>
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<400> 10
aaaagcttta tggaaaggat catgtcgggt cgccgctgc 39

